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13. ABSTRACT ***(Maximum 200 Words)***

This work develops a methodology to assess the acute toxicity induced by the MPTP treatment in a neurotoxic model of Parkinson's disease. Secondly, the progressive loss of dopamine axons can be diagnostically measured by positron emission tomography (PET) and using specific ligands such as CFT. Third, we are addressing whether xenogeneic dopamine neurons can replace the neurons lost by the neurotoxic process. We will also compare neurotransplantation with pallidotomy. The current analysis is dependent upon behavioral, PET, MRI/MRS and finally, post-mortem methodology to determine the questions and objectives outlined in this plan. In this period, we have found through combined PET and MRS studies how neurochemical changes are linked in the primate model for Parkinson's disease. We have obtained specific changes that provide us with predictive mathematical models for the progressive degeneration in Parkinson's disease. These results provide a useful model and novel diagnostic tools for neurotoxically induced Parkinson's disease.

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Table of Contents

INTRODUCTION:

This work develops new functional diagnostics and treatments for Parkinson's disease (PD) from pre-clinical experiments in primate models of neurotoxically induced PD. Given that (1) dopamine (DA) neurons die and a stable PD-like behavioral syndrome appears in primates after chronic administration of a neurotoxin: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), (2) loss of dopaminergic axon can be diagnostically detected by positron emission tomography (PET) and ligands to label striatal DA reuptake sites, (3) neural transplantation may replace neurotoxically eliminated neurons and reverse PD-like symptoms and drug induced side effects, we will now determine how implanted fetal porcine neural DA and control non-DA cells can repair neural systems and reverse behavioral deficits. Pallidotomy is tested as a parallel therapeutic method. We will measure DA receptors and cerebral oxidative glucose metabolism by PET and neuroanatomy, hemodynamics, levels and profiles of brain tissue neurochemicals by MRI/MRS in rodent and primate animal models. The data-sets from PET and MRI/MRS are correlated with behavioral and post-mortem studies. This project develops 1) objective *in vivo* measurements of brain damage associated with neurotoxins and 2) therapies for neurotoxically induced PD.

BODY:

We describe below the research accomplishments associated with the approved Statement of Work, which is copied here in bold. The publications and figures referenced are attached in the Appendix.

STATEMENT OF WORK

WE WILL DETERMINE AND DEVELOP NOVEL DIAGNOSITC CRITERIA FOR ACUTE NEUROTOXICITY AND LONG-TERM DEGENERATION OF THE DOPAMINE SYSTEM (OBJECTIVE 1)

Starting in year 1, and continuing through year 3, we determine in MPTP induced primate parkinsonism, the consequences of acute neurotoxicity (ANT). The following questions are answered in this sequence:

Step 1.1. Are there changes in dopamine reuptake sites or dopamine receptors in ANT?

Our results (Fig. 1) indicate that there is a rapid loss of dopamine reuptake sites and corresponding upregulation of dopamine receptors in ANT. (Year 1 and 2, Brownell et al. 2000, Soc. Neurosci. Abstract, appendix).

Step 1.2. Is there any sign of oxidative stress in ANT?

Our results (Fig. 2, 3) indicate that there are dramatic signs of oxidative stress in ANT, which (see below) continues after chronic loss of dopamine neurons. (Brownell et al. 1998, 1999, 2000, appendix).

Step 1.3. Are there changes in tissue neurochemical profiles in ANT?

Our results indicate that there are initial progressive changes in the neurochemical profile. There are increases in choline and decreases in NAA, there are parallel increases in lactate and macromolecules paralleling Parkinson's disease. (Brownell et al. 1998, appendix). In addition, we have found more evidence of neuroinflammation (Cicchetti et al. 2000, Soc. Neurosci. Abstract, appendix).

Step 1.4. Are there hemodynamic changes observable in ANT?

Yes, see Fig. 4.

Step 1.5. Is there any change in behavioral locomotor activity in ANT?

Our results indicate that there is a rapid loss of locomotor activity which parallels the neurotoxic syndrome. However, this change does not become overt Parkinsonism until a chronic stage of at least 70-80% loss of dopamine. (Brownell et al. 1998, 1999, appendix). Transient or few exposures to MPTP cause transient behavioral dysfunction, but incremental DA fiber loss (Fig. 5A, B, C)

The specific biological questions during longer-term degeneration are studied in years 2-4:

Step 1.6. Does the initial DA loss trigger metabolic and/or neurochemical changes over time in non-DA systems?

Dopamine loss beyond 60-80% in caudate-putamen triggers long-term changes in non-dopamine systems. After MPTP , there are MRI/MRS changes seen two and a half years after the neurotoxin exposure. Similarly, the initial loss of dopamine induces a cascade of degenerations that persist for and terminate years after the initial toxic exposure. (Brownell et al. 1998, 1999, 2000, appendix). Glial or macromolecular MRS changes also normalize after end-stage (severe) MPTP toxicity.

Step 1.7 As an endpoint of stable Parkinsonism longitudinal studies will be correlated with clinically relevant behavior in a slowly progressing primate Parkinson disease model.

These studies are ongoing and indicate that Parkinson's disease is mirrored very closely by MPTP toxin treatment (Brownell et al. 1999, 2000; Isacson et al. 2000).

The PET studies using ^{11}CFT show the binding to dopamine reuptake sites and ^{11}C-raclopride to dopamine D_2 receptors. Oxidative stress was observed by PET studies of oxidative metabolism (oxygen extraction fraction, oxygen metabolism and glucose metabolism) as well as MRS studies of lactate/lipid peaks. MRS studies simultaneously show a number of tissue neurochemicals: choline, creatine, N-acetylaspartate, myo-inositol.

IN EXPERIMENTAL PD MODELS, WE WILL DETERMINE THE MECHANISMS BEHIND EFFECTS OF THERAPEUTIC INTERVENTIONS WITH FETAL NEURONS OR PALLIDOTOMY (OBJECTIVE 2)

Therapeutic interventions will be investigated in combination with PET and MRI/MRS and locomotor activity studies. Initiated in year 1, but continuing through year 4 we will answer the following biological questions in a primate PD model:

Step 2.1. Is there change in dopamine reuptake sites or dopamine receptors after transplantation with DA or non-DA neurons?

One animal has had transplants 2 1/2 months ago. It will receive PET scans using CFT and raclopride.

Step 2.2. Has oxidative stress recovered after transplantation with DA or non-DA neurons?

These studies are in progress.

Step 2.3. Are there changes in tissue neurochemical profiles after transplantation with DA or non-DA neurons?

These studies are in progress.

Step 2.4. Is there vascular arborization after transplantation with DA or non-DA neurons?

These studies are in progress.

Step 2.5.Is there change in locomotor activity after transplantation with DA or non-DA neurons?

These studies are in progress.

Step 2.6. Does pallidotomy effect on regional blood flow, oxygen extraction fraction, oxygen or glucose metabolism?

These studies are in progress (Fig. 6, 7, 8).

Step 2.7. Does pallidotomy have any effect on dopamine reuptake sites or dopamine receptors?

These studies are in progress (Fig. 6, 7, 8).

Step 2.8. Does pallidotomy have any effect on neurochemicals?

These studies are in progress (Fig. 6, 7, 8).

Step 2.9. Does pallidotomy effect on behavioral locomotor activity?

These studies are in progress (Fig. 6, 7, 8).

Step 2.10. The endpoint correlation of parameters derived of imaging studies with behavioral studies and post-mortem histology.

These studies are in progress. Preliminary data indicate that the imaging studies are highly predictive of the postmortem analysis of remaining or degenerated dopamine fibers.

KEY RESEARCH ACCOMPLISHMENTS:

1. The stereotaxic precision surgery using CT-MRI fused imaging and spatial algorithms allow for transplantation and surgical access for the substantia nigra, subthalamic nucleus and globus pallidus (Fig. 7, 8).
2. Acute neurotoxic exposure to MPTP usually has a reversible behavioral outcome (movement analysis) (Fig. 5A, B, C).
3. Repeated neurotoxic exposure caused incremental neuronal damage that progresses to a symptomatic threshold (Brownell et al. 1998, 1999).
4. Progressive but not transient neurotoxin treatment with oxidative stress using complex I inhibitors (toxin: MPTP) creates a syndrome identical to Parkinson's disease in nonhuman primates (Fig. 2, 3, Brownell et al. 1998, 1999, 2000).
5. Repeated neurotoxic treatment with MPTP creates loss of dopamine terminals and a commensurate up-regulation of dopamine receptors (Fig. 1).
6. After PD signs have occurred, magnetic resonance spectroscopy (MRS) shows oxidative stress and neuroinflammation in the striatum long after neurotoxin exposure (Brownell et al. 1999, Cicchetti et al. 2000).
7. Oxidative stress and glial indices eventually normalize after chronic MPTP toxin exposure (Brownell et al. 1999).
8. The loss of dopamine synapses in the caudate putamen after mitochondrial toxin exposure fits an exponential curve and an equation resembling cell survival theory (Brownell et al. 1999).
9. The MPTP primate model has MRI and MRS spectra similar to Parkinson's patients. The predictive value of the equations for this degeneration phenomena provide an opportunity for protective treatments (Brownell et al. 1999).

REPORTABLE OUTCOMES:

Manuscripts:

1. Brownell, A.-L., Jenkins, B.G., Elmaleh, D.R., Deacon, T.W., Spealman, R.D., Isacson, O. (1998) Combined PET/MRS studies of the brain reveal dynamic and long-term physiological changes in a Parkinson's disease primate model. Nature Med. 4, 1308-1312.
2. Brownell, A-L, Jenkins, B. and Isacson, O. (1999) Dopamine Imaging Markers and Predictive Mathematical Models for Progressive Degeneration in Parkinson's Disease. Biomedicine & Pharmacotherapy 53, 131-140.
3. Isacson, O., van Horne, C., Schumacher, J.M., Brownell, A.-L. (2000) Improved surgical cell therapy in Parkinson's disease: physiological basis and new transplantation methodology. In: Parkinson's Disease, Advances in Neurology, D. Calne, ed. Lippincott Williams Wilkins, Philadelphia, PA, in press.

4. Chen, Y.I., Brownell, A.-L., Galpern, W., Isacson, O., Bogdanov, M., Beal, M.F., Livni, E., Rosen, B.R., Jenkins, B.G. (1999) Detection of dopaminergic cell loss and neural transplantation using pharmacological MRI, PET and behavioral assessment. Neuroreport 10, 2881-2886.
5. Costantini, L.C. and Isacson, O.(1999) Dopamine neuron grafts: development and molecular biology. In: Dopamine Neuron Development, U. di Porzio, R. Pernas-Alonso and C. Perone-Capano, eds., R.G. Landes Company, Georgetown, pp. 123-137.
6. Fink, J.S., Schumacher, J.M., Ellias, S.L., Palmer, E.P., Saint-Hilaire, M., Shannon, K., Penn, R., Starr, P., van Horne, C., Kott, H.S., Dempsey, P.K., Fischman, A.J., Raineri, R., Manhart, C., Dinsmore, J., Isacson, O. (1999) Porcine xenografts in Parkinson's disease and Huntington's disease patients: tentative outcomes. Cell Transplant. 9, 273-278.
7. Isacson, O., Costantini, L.C. and Galpern, W.R. (1999) Molecules for neuroprotection and regeneration in animal models of Parkinson's disease. In: Central Nervous System Diseases: Innovative animal models from lab to clinic, D.Emerich, R. Dean and P.R. Sanberg, eds. Humana Press, Totowa, NJ, pp. 187-207.
8. Isacson, O., Costantini, L., Schumacher, J.M., Cicchetti, F., Chung, S. and Kim, K.-S. (2000) Cell Implantation Therapies for Parkinson's Disease Using Neural Stem, Transgenic or Xenogenic Donor Cells. Parkinson's Disease and Related Disorders, Elsevier Science Ltd, in press.
9. Björklund, L., Herlihy, D., Isacson, O. (2000) Cell and synaptic replacement therapy for Parkinson's disease: current status and future directions. Neuroscience News, in press.

Abstracts:

1. A.-L. Brownell, Y.-I. Chen, E. Livni, F. Cicchetti, O. Isacson. Complementary PET studies of striatal dopaminergic system and cerebral metabolism in a primate model of Parkinson's disease. Soc. Neurosci. 2000.
2. O. Isacson. Neural cell transplantation in neurodegenerative diseases. XVIII Intl. Congress of the Transplantation Society, Rome, 2000.
3. A.E. Moore, F. Cicchetti, L. Bjorklund and O. Isacson. Behavioral assays for determining selective dopaminergic degeneration and regeneration in parkinsonian rat models. Soc. Neurosci. 2000.
4. F. Cicchetti, A.L. Brownell, Y.I. Chen, E. Livni, O. Isacson. Neuroinflammation of the nigro-striatal pathway during progressive dopamine degeneration. Soc. Neurosci. 2000.
5. O. Isacson. Development of neuronal repair and reconstruction strategies against neurodegenerative disease. Intl. Workshop on Stem Cell Biology and Cellular Molecular Treatment, Tokyo, 2000.

Presentations:

1999 Austrian Parkinson Society, Vienna (lecture)
1999 Bonn, Intl. Neuroscience Symposium "Molecular Basis of CNS Disorders" (lecture)
1999 London, The Novartis Foundation "Neural Transplantation in Neurodegenerative Disease"
1999 Miami, 6th National Parkinson's Foundation Intl. Symposium on Parkinson's Research (lecture)
2000 Louisville, "The Neuroscience of Developing Cell Therapies for Parkinson's Disease" (lecture)
2000 Zurich, Intl. Study Group on the Pharmacology of Memory, (lecture)
2000 Tokyo, Intl. Workshop: Stem Cell Biology & Cellular Molecular Treatment (lecture)

2000 Il Ciocco, Italy, Gordon Research Conference (lecture)
2000 Rome, Intl. Cong. of the Transplantation Society (lecture)
2000 Turin, Italy, Cellular & Molecular Mechanisms of Brain Repair (lecture)

CONCLUSIONS:

Our transplantation and neurosurgical compensation lesion studies indicate that stereotaxic targeting for reproducible access to substantia nigra, subthalamic nucleus and pallidotomy requires CT-MRI fused imaging and spatial algorithms. Behavioral and PET/MRI studies indicate that low dose exposure to MPTP causes a transient dysfunction, but an incremental neuronal decrease of the dopaminergic system. In vivo PET/MRS brain imaging shows compensatory changes following toxin exposure. The data provide exact mathematical models for which both the degeneration and neuroprotection for PD can be evaluated. Many molecules in the environment and potentially administered toxins can simulate the action of MPTP, which with repeated exposure could increase the risk of Parkinson's disease.

APPENDICES:

Figures 1-8 and Figure Legends

Publications:

1. Brownell, A.-L., Jenkins, B.G., Elmaleh, D.R., Deacon, T.W., Spealman, R.D. and Isacson, O. (1998) Combined PET/MRS brain studies show dynamic and long-term physiological changes in a primate model of Parkinson disease. Nature Medicine 4, 1308-1312.
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5. Björklund, L., Herlihy, D., Isacson, O. (2000) Cell and synaptic replacement therapy for Parkinson's disease: current status and future directions. Neuroscience News, in press.
6. A.L. Brownell, B.G. Jenkins, D.R. Elmaleh, T.W. Deacon, O. Isacson, Long-Term In Vivo PET/MRS Neurodegeneration Studies of a Primate Parkinson's Disease Model, Soc. Neurosci. 1998.
7. A.-L. Brownell, T. van Nguyen, Y-C. J. Chen, F. Cavagna, B.R. Bosen, O. Isacson, B.Q. Jenkins. PET and phMRI sutides of dopamine receptor modulation in PD models. Soc. Neurosci. 1999.
8. A.-L. Brownell, Y.-I. Chen, E. Livni, F. Cicchetti, O. Isacson. Complementary PET studies of striatal dopaminergic system and cerebral metabolism in a primate model of Parkinson's disease. Soc. Neurosci. 2000.
9. F. Cicchetti, A.L. Brownell, Y.I. Chen, E.Livni, O. Isacson. Neuroinflammation of the nicro-striatal pathway during progressive dopamine degeneration. Soc. Neurosci. 2000.

Curriculum Vitae: Dr. Ole Isacson

FIGURE LEGENDS:

Figure 1:

Dopamine receptors supersensitivity during MPTP in primate brain

PET imaging of dopamine transporter binding was performed using ^{11}C-CFT and D_2 receptor binding using ^{11}C-raclopride. Top panel illustrates control studies conducted before MPTP treatments for both dopamine transporter and D_2 receptor binding in 3 coronal sections at 20, 15 and 10mm, anterior to the ear level. Lower panel depicts decreased dopamine transporter binding after acute MPTP treatments and elevated binding of D_2 receptors post-treatment.

Figure 2:

Oxygen metabolism during acute MPTP in primate brain

Oxygen metabolism was investigated using a steady state inhalation technique of $^{15}O_2$-gas. Six coronal sections illustrate the magnitude of the metabolism distribution at two time points after acute MPTP treatments with one month interval between measurements. The results show a significant increase in oxygen metabolism one month after acute MPTP treatments. Note that the color coding may not reflect absolute change.

Figure 3:

Glucose metabolism during acute MPTP in primate brain

Glucose metabolism was investigated using ^{18}F-labelled flurodeoxy glucose. Six coronal sections illustrate the magnitude of the metabolism distribution before and during the acute MPTP treatment with one month interval between measurements. The experiments illustrate an initial reduction in glucose metabolism and normalization after one month.

Figure 4:

Blood flow during acute MPTP in primate brain

Blood flow was investigated using a steady state inhalation technique of $C^{15}O_2$-gas. Six coronal sections illustrate the magnitude of the flow distribution at two time points during the acute MPTP treatment with one month interval between measurements. The images reveal an increased blood flow evident one month after the MPTP treatment.

Figure 5:

Locomotor activity prior to and during acute MPTP treatments

Graphs illustrating mean locomotor activity prior to and during acute MPTP treatments on *maccaca fascicularis,* a) #463.97, b) 458.97 and c) 464.97 over several weeks of observation. Data for daytime and nighttime activity, collected by the actiwatch activity monitor, are shown. Black arrows indicate weeks of MPTP treatments. The actiwatch activity monitor is a small

device held in the pocket of a jacket worn by the animal for the duration of the experiment. The monitor measures momentum change.

Figure 6:

Photograph of the pallidotomy procedure on the maccaca fascicularis #463.97

Side-view of the animal in the human head frame with electrode attachment in place for single-unit recording. Recordings were performed to confirm globus pallidus target sites selected on the Radionics stereoplan system shown in Figure 7.

Figure 7:

Stereoplan imaging system

Screen print of Radionics stereoplan imaging system used for the targeting of the brain sites prior to the surgical procedure. Top left panel depicts an axial plan of the *maccaca fascicularis* #463.97 brain at a dorso-ventral level where targeted sites are clearly visible. The small cross on the monkey's right globus pallidus marks the selected area for lesion. Top right panel shows a table of the coordinates used to determine the optimal lesion site. The fourth set of coordinates were used for lesioning. Bottom panel depicts coronal (left) and sagittal (right) brain sections after selection of the specific points for targeting. The pallidotomy procedure included a single-unit recording session to confirm selected coordinates. After confirmation, the lesion electrode was inserted into the brain starting at 10mm above target to the exact target zone. At 2mm increments, hand movements generated by stimulation at different voltages were observable. At target site, hand movements were generated at ideal low voltage stimulation and heat lesion (70 degrees) for 60 seconds was performed.

Figure 8:

Stereoplan imaging system

High magnification of the axial plan (shown in Figure 8) of the animal's brain at a dorsoventral level where targeted globus pallidus sites are clearly visible.

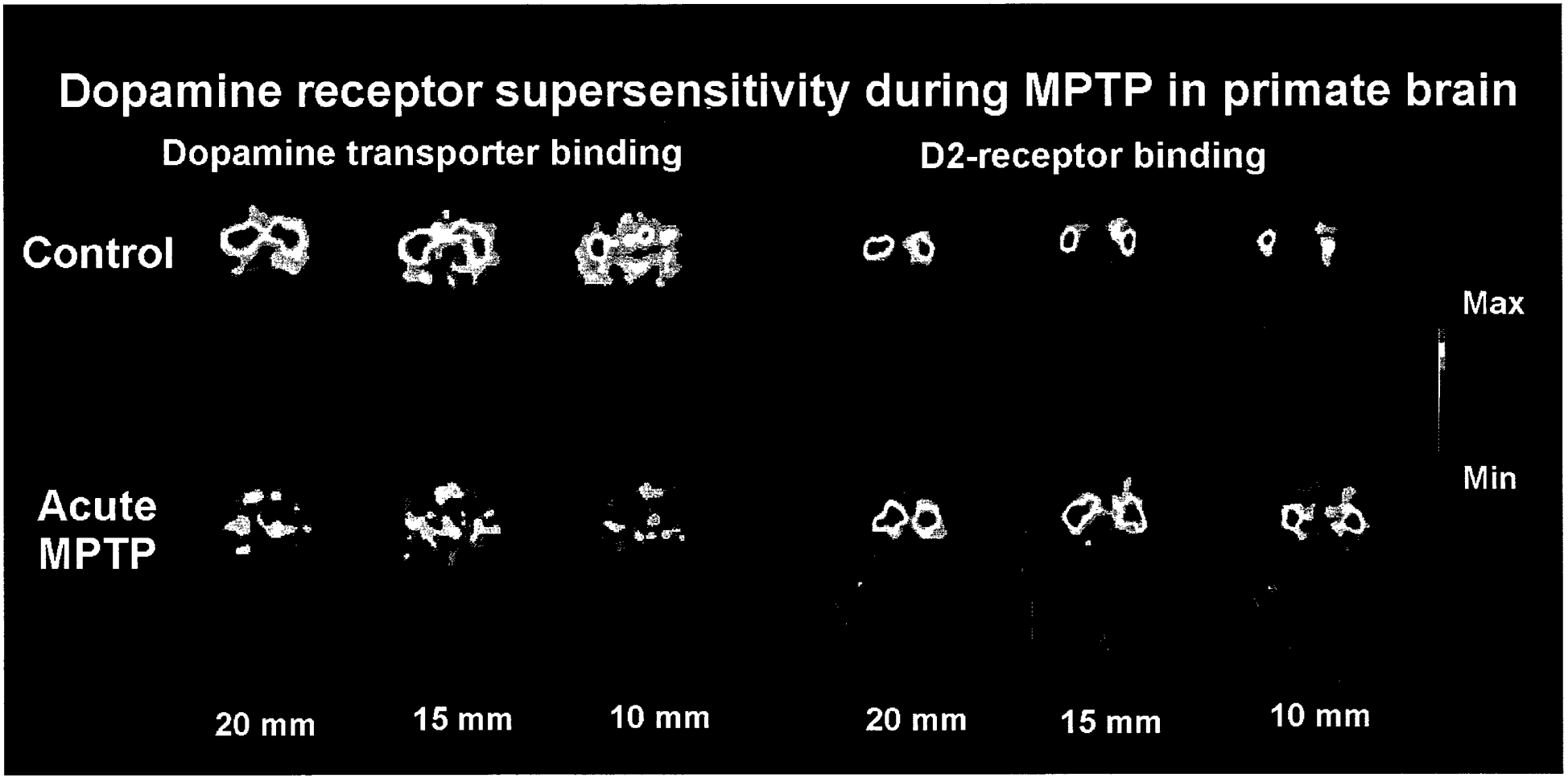
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Figure 1

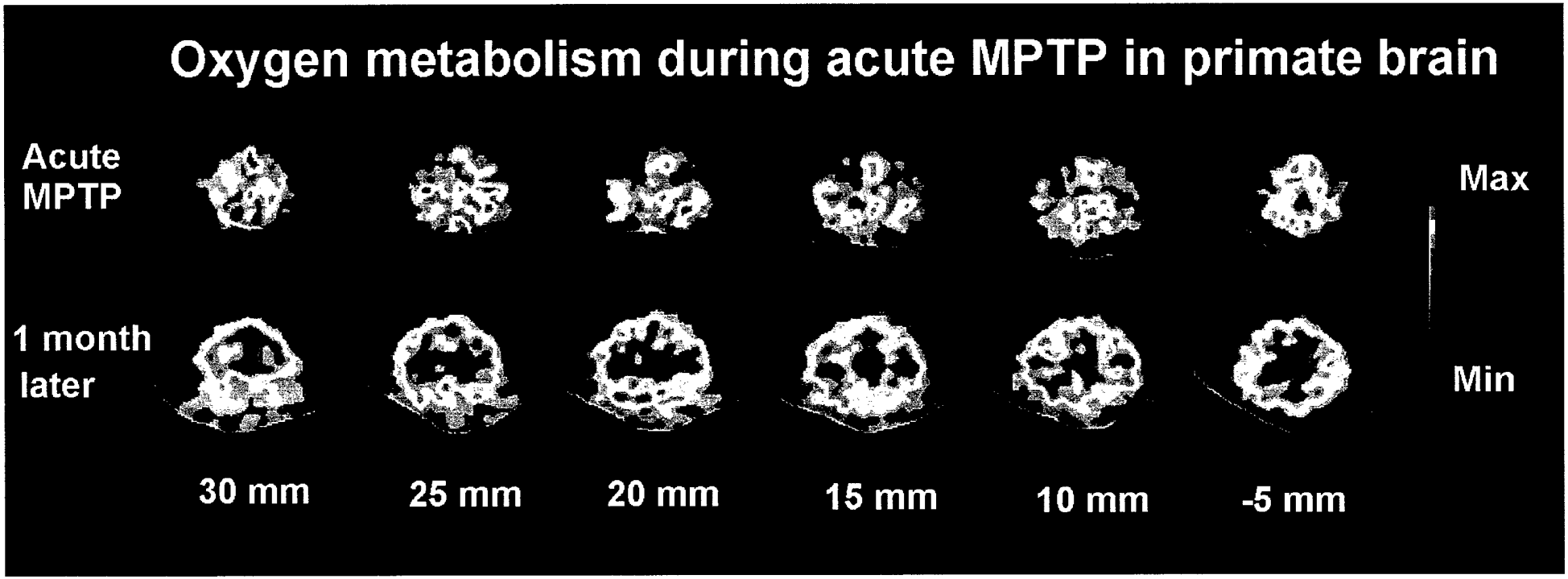
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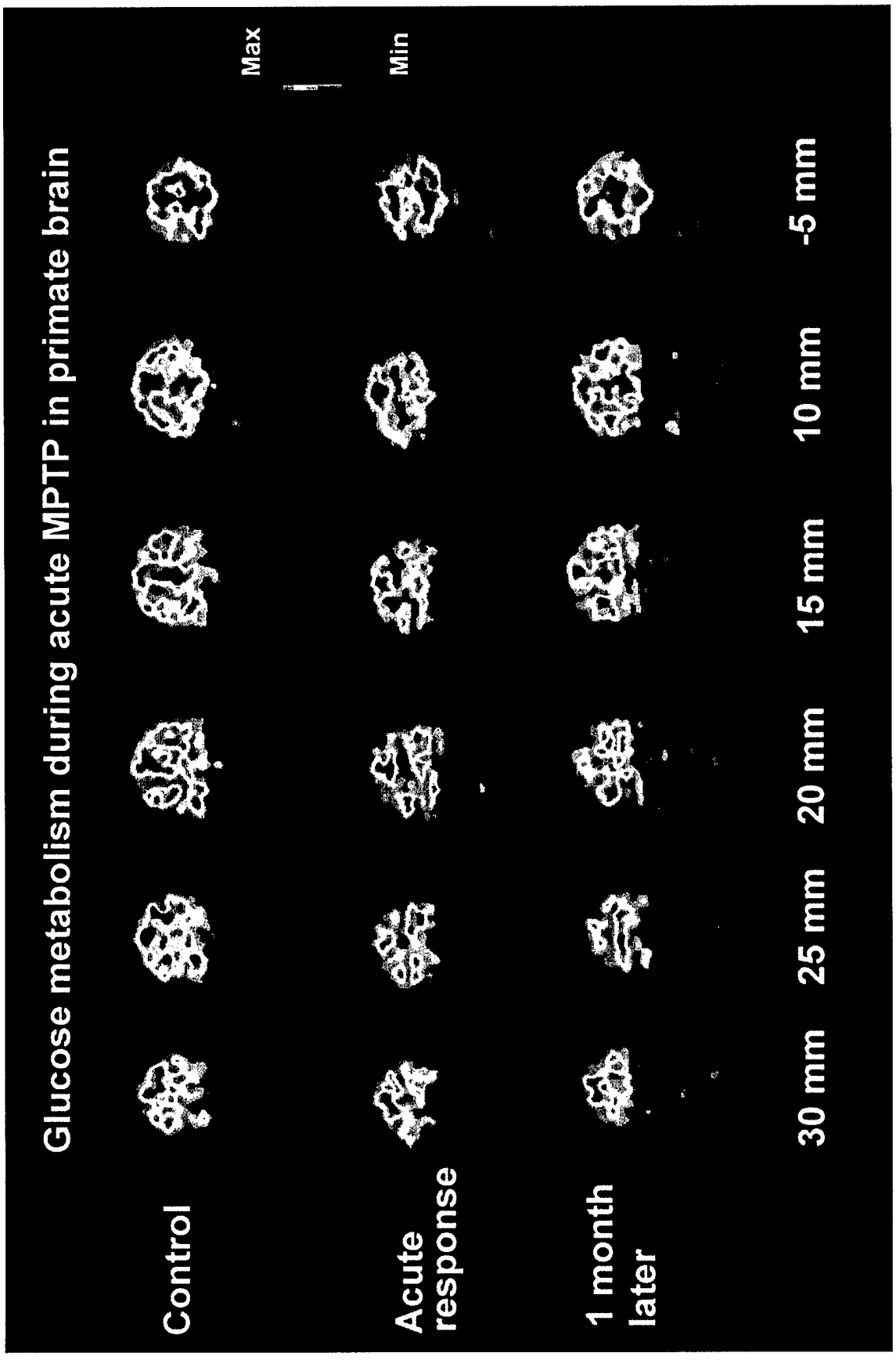
Figure 2

Figure 3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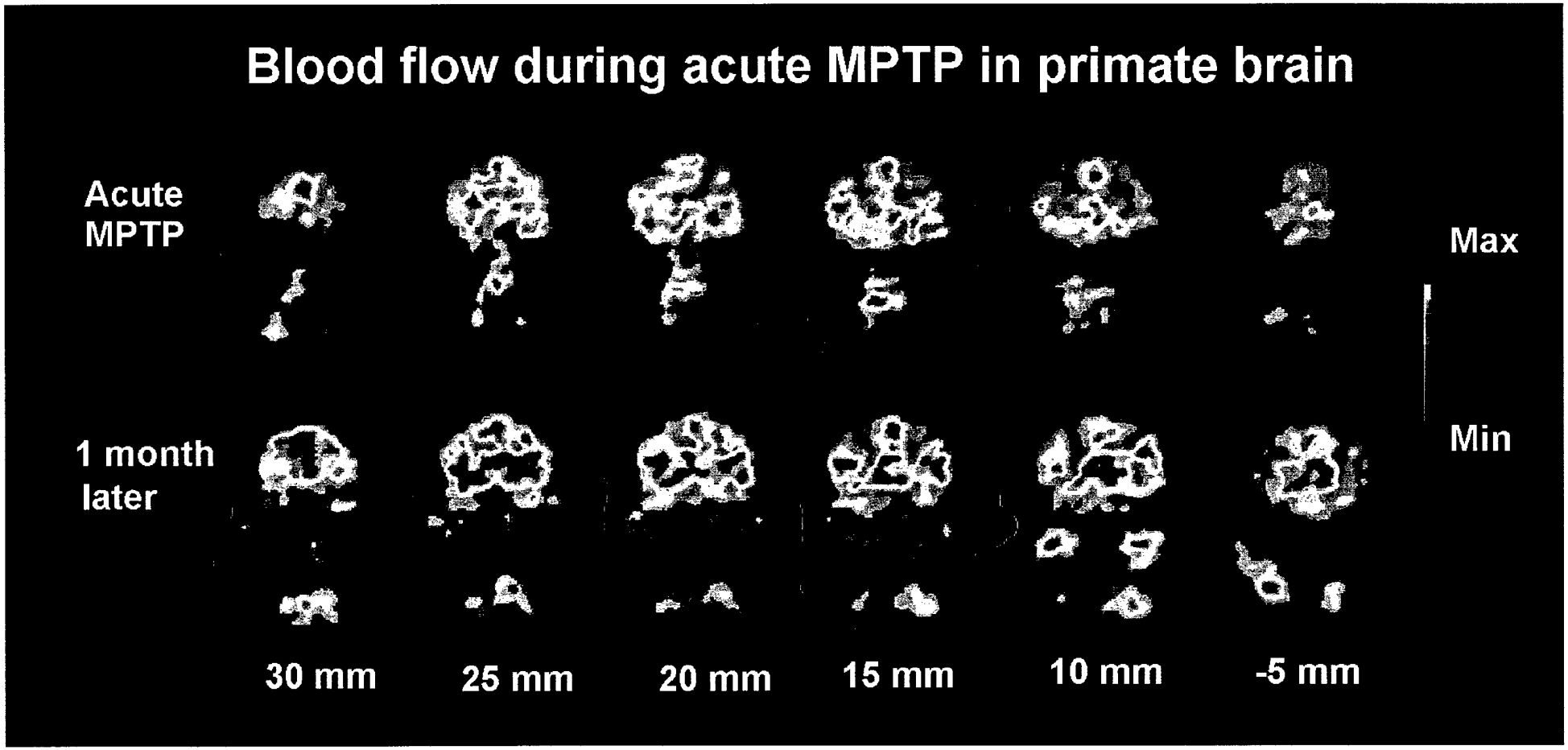
Blood flow during acute MPTP in primate brain
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Figure 4

Figure 5A

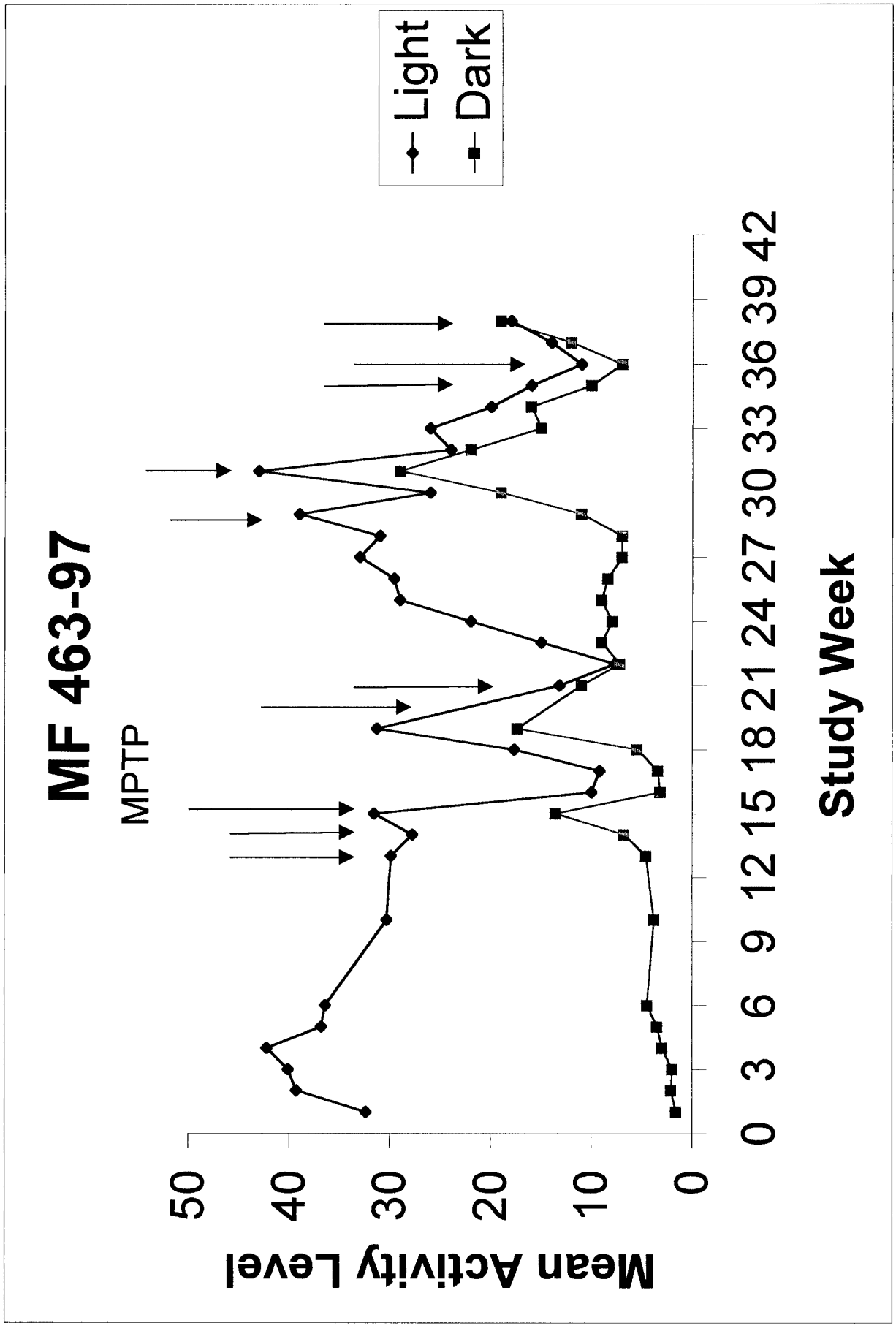
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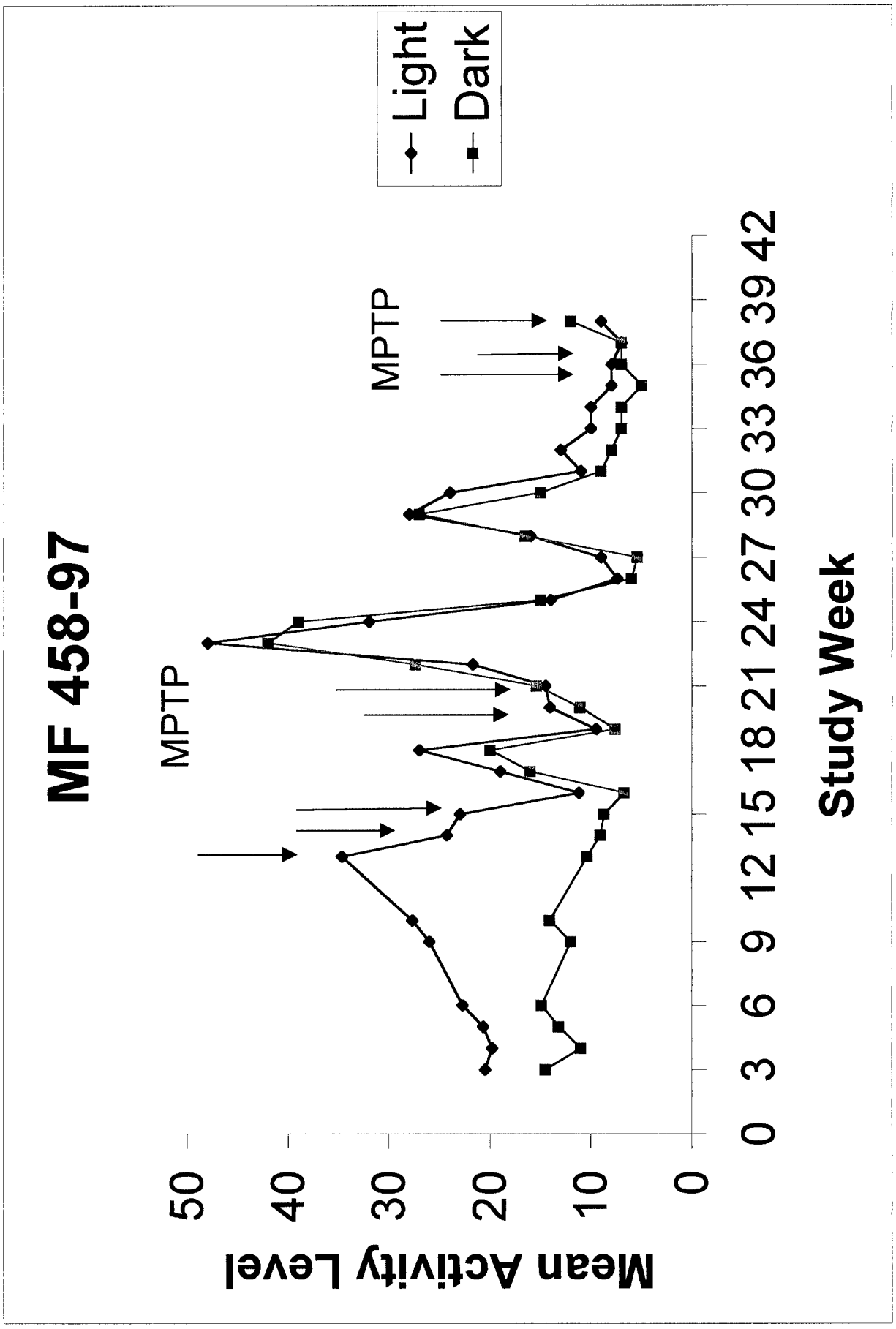
Figure 5B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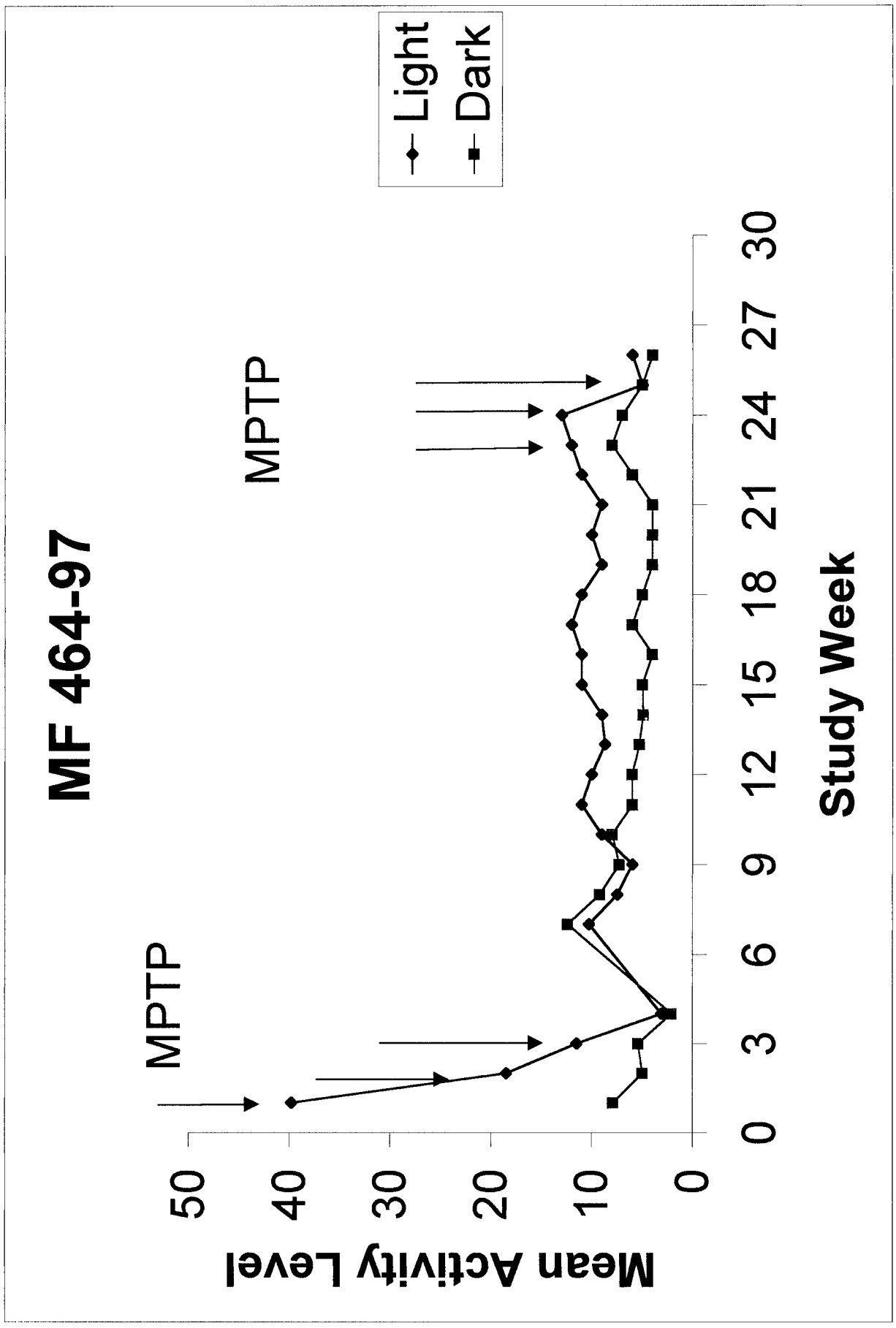
Study Week

Figure 5C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Study Week

Figure 6

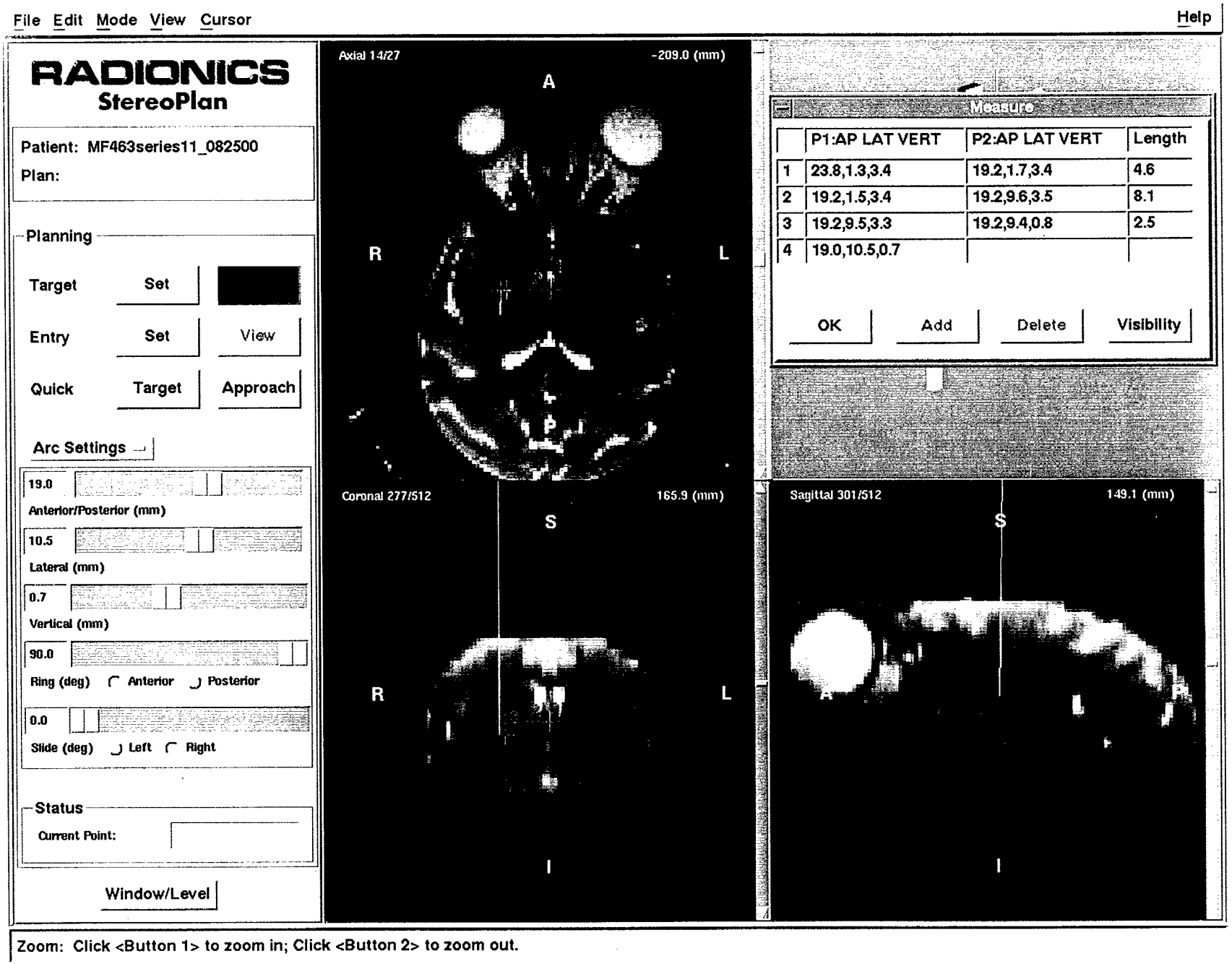
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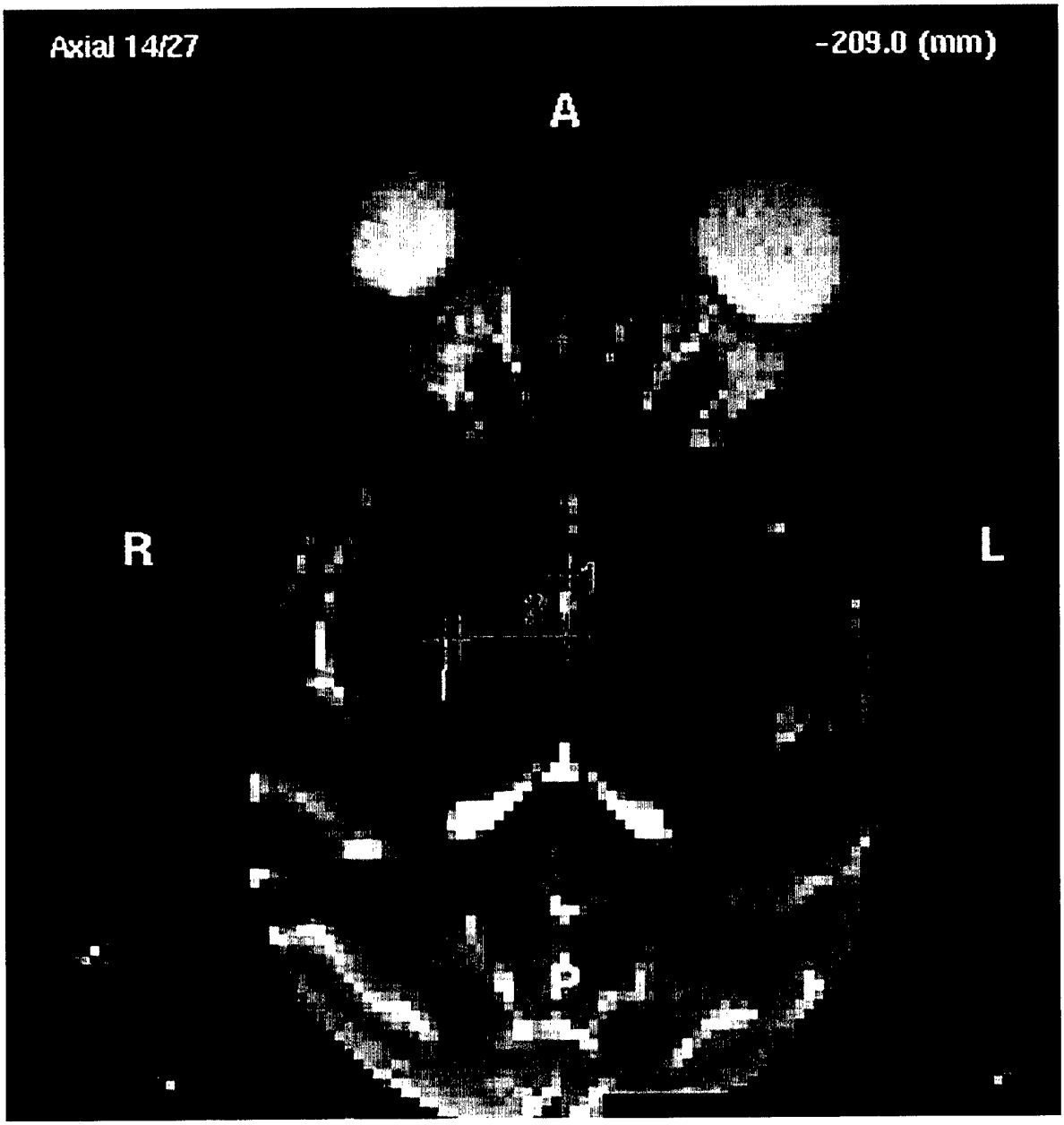
Figure 7

Figure 8



Combined PET/MRS brain studies show dynamic and long-term physiological changes in a primate model of Parkinson disease

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We used brain imaging to study long-term neurodegenerative and bioadaptive neurochemical changes in a primate model of Parkinson disease. We gradually induced a selective loss of nigrostriatal dopamine neurons, similar to that of Parkinson disease, by creating oxidative stress through infusion of the mitochondrial complex 1 inhibitor MPTP for 14 ± 5 months. Repeated evaluations over 3 years by positron emission tomography (PET) demonstrated progressive and persistent loss of neuronal dopamine pre-synaptic re-uptake sites; repeated magnetic resonance spectroscopy (MRS) studies indicated a 23-fold increase in lactate and macromolecules in the striatum region of the brain for up to 10 months after the last administration of MPTP. By 2 years after the MPTP infusions, these MRS striatal lactate and macromolecule values had returned to normal levels. In contrast, there were persistent increases in striatal choline and decreases in N-acetylaspartate. Thus, these combined PET/MRS studies demonstrate patterns of neurochemical changes that are both dynamic and persistent long after selective dopaminergic degeneration.

In neurological diseases like Parkinson disease (PD), examination of the living brain by high resolution positron emission tomography (PET) and magnetic resonance imaging (MR), combined with the appropriate pharmaco-kinetic and physiological analyses, can provide valuable quantitative information of altered brain function[1,2]. Imaging technology depends on the limits of imaging (resolution and sensitivity) as well as biological variables (tissue structure and biochemical processes)(refs. 3–6). In applications involving the human brain, recent progress in obtaining localized magnetic resonance spectra (MRS) and spectroscopic images has made possible new studies of tumors[7,8] and infarcts[9,10], as well as examination of normal brain physiology[11].

The most prominent pathological change in idiopathic Parkinson disease is degeneration of the nigrostriatal-dopaminergic pathway associated with severe cell loss in the substantia nigra[12]. In patients, a chief consequence of the loss of dopamine (DA) neurons is a substantial decrease in the density of dopaminergic synapses and in the concentrations of DA in the striatum[13,14]. The striatal loss of DA results in typical signs, including akinesia, bradykinesia, rigidity and resting tremor. These findings led to experiments aimed at developing animal models of PD using neurotoxins; such as 6-hydroxydopamine[15,16], selective for DA neurons. Some cases of parkinsonism have developed after accidental intravenous self-administration of a meperidine analogue; 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine[17] (MPTP). The affected individuals had symptoms that included severe akinesia, rigidity, flexed posture and a resting tremor. The symptoms were associated with decreased striatal ^{18}F-fluoro-L-dopa uptake, observed using PET[18], and considerable loss of pigmented neurons in the substantia nigra.

In primates, administration of MPTP by stereotaxic application in the striatum, intra-carotid injections or repeated intravenous injection over 5–10 days[19–21] generally induces a substantial DA depletion resulting in a severe akineto-rigid PD syndrome (often requiring drug therapy) within weeks after administration of the neurotoxin. In contrast, repeated low-dose administration of MPTP over a longer period of time (up to 19 months) increases the selectivity of the neurotoxin for specific subpopulations of DA neurons, more accurately reproducing the pattern of neuropathological and neurochemical alterations observed in idiopathic PD[22,23]. In this chronic administration model, and in idiopathic PD[5,24], signs develop gradually, and after these signs appear they do not spontaneously recover as reported in some acute MPTP models[25]. This animal model therefore represents a stable parkinsonian syndrome, which is necessary for the exploration of long-term functional changes and experimental therapies.

The ability of MRS to sensitively measure neurochemicals in brain volumes less than 1 ml provides a unique 'window' into neurodegenerative processes. MRS is especially useful because it allows quantification of different chemicals in a single study, which can be repeated many times. Chemicals quantifiable in proton MRS include N-acetyl aspartate (NAA), a correlate marker for healthy mature neurons[26,27]. Thus, MRS has been used to study neuronal loss, using NAA as a marker[28–31]. Loss of NAA may not always correlate with the final destruction of

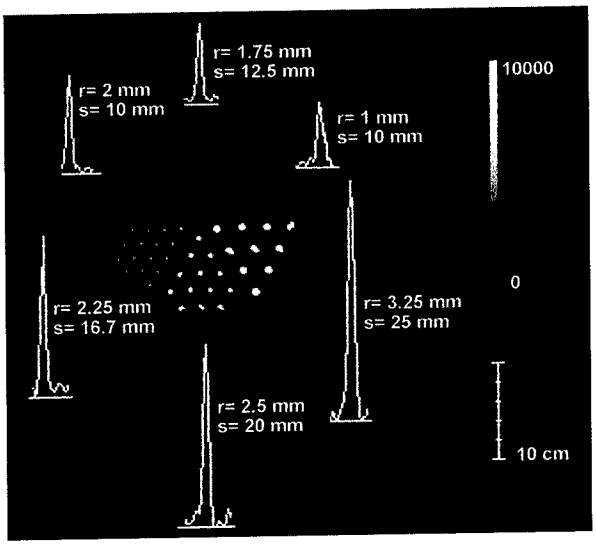
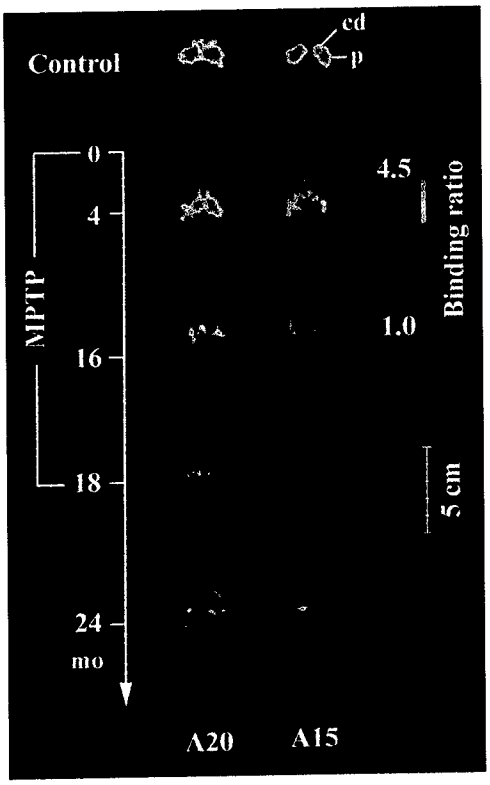


Fig. 1 PCR-I with ^{18}F-labeled water in a 'Derenzo-phantom'. PCR-I is a high-resolution brain imaging device; the phantom is a solid plastic disk with six sectors of holes each of a different radius (r) and separations (s). There is uniform distribution of radioactivity in all the sectors, with clearly separated images even for holes 2.0 mm in diameter with 10-mm separation. Spectra next to each sector describe measured count distribution in a single hole in each sector corresponding a volume of $3.14 \times (r)^2 \times 5$ mm^3 (the thickness of the slice is 5 mm). Scale bar represents 10 cm, with each division being 2 cm.

neurons, but to some degree may reflect their health[32,33]. In addition to NAA, substances such as lactate, glutamate, creatine, choline and myo-inositol provide a view of the progression of neurodegeneration; for example, in gliosis, glial cells have a concentration of cholines (trimethylamines) twice that of neurons[27]. Elevated choline concentrations are also found in conditions involving the proliferation of pathological forms of glial cells such as gliomas[7,8]. The main limitation in using MRS is its relative insensitivity compared to PET, because of the low signal obtained per molecule. NAA, the most prominent molecule in a brain proton spectrum, has an approximate concentration of 8–10 mM in the brain. Even at this concentration, MRS yields a low signal-to-noise ratio, which leads to a relatively low spatial resolution. Recent developments in PET instrument design have greatly improved the performance of PET[34,35]. Theoretically, the resolution of PET is limited by three factors: positron range, small angle deviation, and the sampling achieved by the detectors. For these experiments, positron emission tomography studies were done using a PET scanning system (PCR-I) equipped with one ring of 360 BGO (bismuth germanate) detectors and a computer controlled imaging table[36]. Here we have studied the long-term physiological changes after MPTP-induced neurotoxicity using PET and MRS techniques, in a primate model of PD.



Functional PET studies

Using a specially adapted PET scanning system (Fig. 1), we investigated chronic neurodegenerative processes over 3 years in a Parkinson disease model in five cynomolgus monkeys (Macaca fascicularis). We used carbon-11-labeled 2β-carbomethoxy-3β-(4-fluorophenyl) tropane (^{11}C-CFT, or WIN 35,428) as a tracer for visualizing dopamine re-uptake sites located on presynaptic dopamine terminals in experimental animals. We compared regional accumulation of ^{11}C-CFT in the striatum at two different coronal brain levels (A20 and A15 from the stereotaxic zero) with its accumulation in the cerebellum in the weeks before, during and after administration of MPTP; this treatment produces a parkinsonian brain degeneration of the dopamine system (Fig. 2). The striatal-to-cerebellar ratio of the ^{11}C-CFT accumulation was 4.5 in the pre-MPTP study and declined with the onset of MPTP administration. Spontaneous locomotor activity decreases in parallel with the decline of the ^{11}C-CFT uptake[23]; however, overt Parkinsonian signs appear only after locomotor activity and the ^{11}C-CFT uptake rate decline to about 30% of their pre-MPTP values[23]. Here the putaminal binding potential of ^{11}C-CFT continues to decline 5–8 months after termination of MPTP administration (Fig. 2) and remains at this level for 2 years after MPTP treatment (Fig. 3). Similarly, the ^{11}C-CFT levels in caudate continued to decline from 55% when MPTP treatment was stopped (Fig. 2) to 21 ± 9% 5–8 months after its termination, and remained at this level for 2 years (Fig. 3). Thus, functional degeneration of DA terminals continues for approximately 5–8 months after MPTP treatment ends and then does not spontaneously recover. During MPTP administration, ^{11}C-CFT accumulation decreased at a faster rate in putamen than in caudate (as seen in PD)(Figs. 2 and 3), indicating that DA terminals are more sensitive to MPTP in the putamen than in the caudate.

MRS studies during neural degeneration

We used ^{1}H water-suppressed MRS to measure biochemical changes in the striatum during MPTP-induced neurodegenera-

Fig. 2 A long-term follow-up study of comparative distribution of ^{11}C-CFT in a primate Parkinson disease model. Two representative coronal brain levels (A20 and A15) are presented before (0), during (4,16,18) and 6 months after (24) MPTP treatment. Images are normalized to cerebellar activity and represent distribution of specific to nonspecific binding of ^{11}C-CFT in the brain 60–62 min after administration of the labeled ligand.

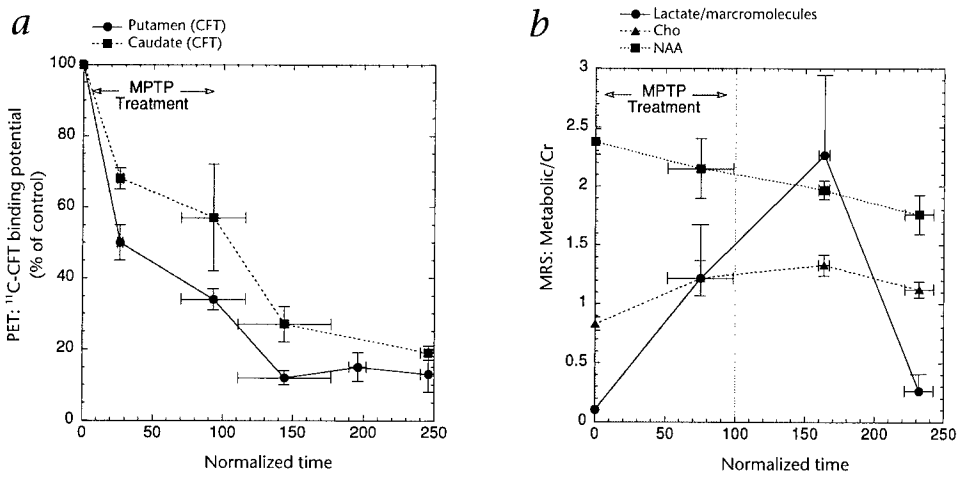


Fig. 3 PET studies of ^{11}C-CFT binding (***a***) and MRS studies of striatal biochemistry (***b***) before, during and after MPTP-induced neurotoxicity. There were irreversible changes of ^{11}C-CFT binding, choline and N-acetylaspartate concentration, as well as a 23-fold increase in peaks corresponding to lactate and macromolecule concentration that was reversible. Normalized time scale (horizontal axis) is obtained based on the response to MPTP-induced neurotoxicity in individual monkeys (as in patients, susceptibility varies). When the monkey showed overt parkinsonian symptoms, MPTP was terminated. The time of the MPTP treatment was normalized to 100, and the follow-up period was also normalized for each animal according this scale. The control value of the binding potential (k_3/k_4) was normalized to 100 and all the follow-up values were also normalized using this scale. The average follow-up time post MPTP was 2 years and the average value of the binding potential in control studies was 4.6–5.6 in putamen and 4.8 –6.6 in caudate region of the striatum.

tive processes. Complementary studies of DA re-uptake sites by PET and neurochemical changes by MRS are shown before MPTP treatment and 2 months after the last MPTP administration (Fig. 4). MPTP induced elevation of lactate/macromolecules and choline peaks (Figs. 3 and 4). Even as much as 10 months after termination of MPTP-induced neurotoxicity, the elevation in lactate/macromolecular peak was 23-fold ± 7-fold (Fig. 3). The choline/creatine (Cho/Cr) ratio in control monkeys was 0.83 ± 0.06 (Fig. 4), whereas it was 1.30 ± 0.15 in the 8–10 months after MPTP-induced neurotoxicity (Figs. 3 and 4). The NAA/Cr ratio in the control monkeys had very high inter-animal reproducibility (2.38 ± 0.11). This ratio decreased slightly but significantly in MPTP-treated monkeys to 1.93 ± 0.21 ($P < 0.01$) in the striatum 8–10 months after termination of MPTP treatment. This finding may reflect that MPTP is mostly neurotoxic for dopaminergic neurons in the substantia nigra, with only transsynaptic anterograde degeneration of the striatum[37,38]. Our data also show that the changes in NAA and Cho persisted after MPTP-induced neurotoxicity (Fig. 3). Two years after MPTP treatment stopped, the increase of choline in treated monkeys was 38 ± 4 % of the control value, and the corresponding decrease of NAA was 26 ± 4 % (Fig. 3). In contrast, the changes in lactate /macromolecular signal are reversible; by 2 years after final MPTP administration, this value had returned to control (background) levels. At approximately the time the striatal level of DA reuptake sites (^{11}C-CFT) reached a minimum in PET studies, the lactate peak seen with MRS reached a maximum.

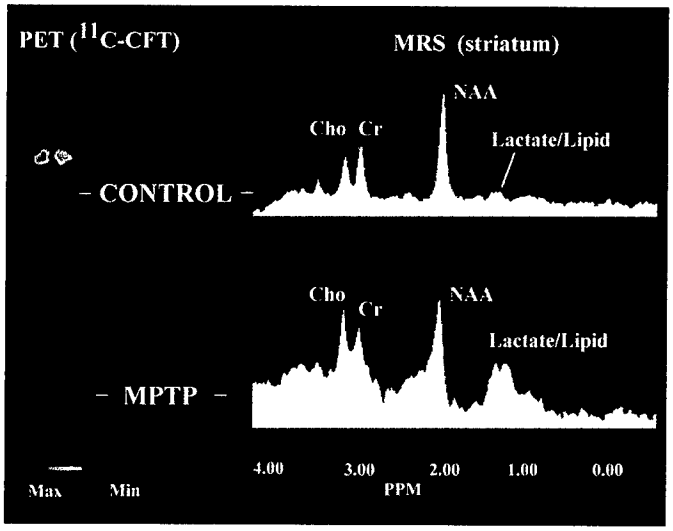


Fig. 4 PET and MRS studies of a monkey before any MPTP and 2 months after the last MPTP treatment. PET images (left) demonstrate that specific/nonspecific binding ratio of ^{11}C-CFT was considerably decreased after MPTP treatment (color coded by Max–Min bar at bottom). MRS (right) demonstrates a decreased NAA/Cr ratio, an elevated Cho/Cr ratio and an elevated lactate and macromolecule peak after MPTP treatment (TR/TE 2000/272 ms; PRESS).

Discussion

These experiments demonstrate, through the combined use of PET and MRS methods, a dynamic and specific neurochemical pattern of long-term neurodegenerative changes in the primate striatum after DA loss similar to that of PD. The physiological changes characterized by this combined PET/MRS approach provide data for a comprehensive *in vivo* analysis of the ongoing biological processes occurring after selective neural degeneration.

In animal models[22,23] and in humans[39], ^{11}C-CFT is a useful ligand to monitor DA terminal degeneration by PET scanning[23]. CFT was the first ligand to demonstrate a loss of DA fiber density equivalent to the loss of DA in human post-mortem Parkinson-diseased brains[40]. ^{11}C-CFT binding also correlates with motor signs in the MPTP primate model of Parkinson disease[22]; these observations have been verified in a larger series of primates[23] and are analogous with findings in early Parkinson disease in humans[40].

Here we studied ^{11}C-CFT levels and biochemical parameters in the striatum of each monkey for about 2 years after the monkey developed overt parkinsonian signs (at which time MPTP treatment was terminated). These data show persistent long-term physiological changes in striatal CFT binding and MRS-identified levels of choline and NAA. The changes in NAA and

choline levels were moderate and are consistent with an interpretation that MPTP-induced neuronal loss is mostly in the substantia nigra and that transsynaptic anterograde degeneration is in striatum[37,41]. MRS studies in patients with idiopathic Parkinson disease show few changes in striatal NAA or choline, but decreases of NAA and increase of choline are seen in some forms of parkinsonism[42,43].

We noted large changes in the MR spectral region between 1 ppm and 1.5 ppm (corresponding to lactate and macromolecules). Before the oxidative stress induced by MPTP, the intensity of this spectral band in the striatum was at background level, but with MPTP treatment, several large selective increases in striatal signal intensity were observed. First, there was a large increase of intensity at 1.33 ppm consistent with elevations in lactate (Fig. 3). After termination of MPTP treatment, there were even larger increases in signals at both 1.0 and 1.3–1.5 ppm. These later changes may reflect ongoing oxidative stress caused by physiological adaptive changes in function of the striatum. The presence of large amounts of mobile lipids acutely after MPTP treatment provides evidence for neuronal membrane breakdown possibly caused by lipid peroxidation or cell death mediated through cellular respiratory-chain inhibition[10,38]. However, the molecular species involved have not yet been specifically identified[10,38]. Detailed histological analysis of the striatum, however, indicates very little macrophage infiltration or gliosis in the MPTP-treated striatum in this progressive MPTP-induced degeneration[20,22]. Nonetheless, minor local striatal neuronal loss around large blood vessels and arterioles has been observed (O.I. and N.K. Kowall, unpublished observation), probably a consequence of direct MPTP-induced neuronal degeneration and mild gliosis from high toxin levels next to blood vessels (from intravenous administration of MPTP). The changes in the lactate and macromolecular peaks are reversible, however, and return to baseline 2 years after termination of MPTP administration.

These dynamic neurochemical shifts that occur several years after the neurotoxic event may relate to important physiological and pathological processes. For example, the signs of PD are not discernable in a patient until there is a 60–80% decrease in striatal dopamine levels. This in itself indicates fundamental adaptive physiological processes that maintain striatal function despite considerable degeneration of one transmitter system. Beyond this critical threshold, PD unfolds in a movement disorder that can, at least initially, be reversed by DA drug replacement therapy. Because the results of the MPTP treatment used here closely resemble the DA degeneration seen in PD, the movement disorder in this primate model also develops at the critical threshold of DA loss[22,23]. The dynamic and persistent physiological changes seen here using PET and MRS may therefore reflect similar adaptive striatal responses to those occurring in PD. Furthermore, the oxidative stress seen years after the neurotoxic events leading to DA loss indicate that the striatal neuronal circuitry may be compromised and at risk for subsequent structural and pathological processes. Future investigations should determine if such physiological stress of the caudate-putamen also occurs after other types of neurodegenerative events, or after long-term pharmacologically induced changes of the DA system[44].

These data indicate that the structural and neurochemical changes after a DA neurotoxic event are dynamic and complex, and continue to develop long after the neurodegenerative stimulus has stopped and PD signs develop. The characterization of these physiological changes may provide insights and a time frame for new therapeutic interventions in PD.

Methods

Primate model. The behavioral model of PD in cynomolgus monkeys (Macaca fascicularis) was produced by the chronic administration (0.6 mg/kg intravenously, every 2 weeks until behavioral stability) of the mitochondrial complex 1 inhibitor 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine[23] (MPTP). Spontaneous locomotor activity was quantified by continuous monitoring with four pairs of infrared motion detectors. Additional video recording and assessment was done monthly. Hypokinesia (decreased frequency of spontaneous movement), bradykinesia (slowness of movement) and tremor were rated by two independent observers to generate a clinical score (0–12), as reported[23]. Animals used in this study were maintained according to the guidelines of the Committee on Animals of the Harvard Medical School and Massachusetts General Hospital and those of the *Guide for Care and Use of Laboratory Animals* of the Institute of Laboratory Animal Resources, National Research Council, Department of Health, Education and Welfare.

PET techniques. The resolution of PCR-I for a point source at the center is 4.5 mm, and the sensitivity is 46,000 counts per s for a source 20 cm in diameter with a concentration of 1 μCi/ml. The overall detection efficiency of photons is 64% of the theoretical maximum for a plane thickness corresponding to the 2-cm-high detectors. A plane thickness of 5 mm (as used in this study) is obtained by limiting the effective height of detectors with cylindrical collimators, and it corresponds to a volume resolution of 0.08 ml. The resolving time of the PCR-I is 6 ns (FWHM).

We used a 'Derenzo-phantom' initially, with ^{18}F-labeled water as a radioactive tracer (Fig. 1). The phantom is a solid plastic disk with six sectors of holes of different diameters and separations. All the holes have the same length (25 mm). The smallest holes have a diameter of 2.0 mm and the separation between the midpoints of the holes is 10 mm. The largest holes have a diameter of 6.25 mm and the separation between the midpoints of these holes is 25 mm. Using the PCR-I, it is possible to image objects 2 mm in size separated by 1 cm (Fig. 1).

The synthesis of ^{11}C-CFT involves direct ^{11}C-methyl iodide methylation of 2β-carbomethoxy-3β-(-4-fluorophenyl)tropane (WIN 35,428; prepared by Organix, Woburn, Massachusetts) as published[45]. For PET imaging, monkeys were anaesthetized with 30mg/kg ketamine and 3mg/kg xylazine (initial dose, intramuscularly), and anesthesia was maintained with half this dose as needed. The femoral artery and vein were catheterized for collection of blood samples and injection of labeled ligand. The monkey was placed in the imaging position and the head was adjusted in a stereotaxic headholder with the earbar as a reference plane. Interior orbital supports ensure that images are acquired in pseudocoronal plane perpendicular to the orbito–meatal line. This allows superposition of data from MRI and MRS studies. After administration of labeled ligand (5 mCi; specific activity 600–1000 mCi/μmol) into the femoral vein, imaging data were collected 'stepwise' on seven coronal levels: A30 (that is, 30 mm anterior from the earbar), A25, A20, A15, A10, P5 (that is, 5 mm posterior from the earbar) and P10. The initial acquisition time per image was 15 s; it was subsequently increased to 60 s with the total imaging time being 90 min. Eighteen arterial blood samples of 0.1 ml each were drawn to monitor the decrease of radioactivity, starting a frequency of 15 s and ending with a frequency 15 min. In addition, six arterial samples were collected for HPLC analyses of metabolites of labeled ligand. Calibration of the positron tomograph was done for each study session, using the cylindrical plastic phantom (diam. 6 cm) and ^{18}F-labeled water. Cross-calibration with a gamma counter (Cobra Auto-gamma; Packard, Downers Grove, Illinois) was also done using ^{18}F-labeled water. Plasma data were corrected for counting efficiency, calibration factor and measured metabolites, and percent activity of the injected dose and ligand concentration were calculated. Imaging data were corrected for uniformity, sensitivity, attenuation, decay and collection time. PET images were reconstructed using Hanning weighted convolution backprojection[46]. Regions of interest (including left and right caudate and putamen, frontal cortex and cerebellum) were outlined from anatomical representations on the screen, and activity per unit volume, percent activity of the injected dose and ligand concentration were calculated. Data were analyzed using a three-compartmental model[39] and

SAAM program[47]. Plasma data were corrected for metabolites using an experimental two exponential correction function; $f(t) = 0.709 \times \exp(-0.108 \times t) + 0.286 \times \exp(-0.014 \times t)$. Binding potential was calculated as a ratio of transportation coefficients (k_3/k_4) into (k_3) and from (k_4) the area of interest (caudate or putamen).

MRS techniques. Monkeys were scanned on a GE 1.5T Sigma scanner (General Electric, Milwaukee, Wisconsin) using a saddle coil 15 cm in diameter. Monkeys were anesthesized with a dose of a mixture of 30 mg/kg ketamine and 3mg/kg xylazine. In the neurochemical analysis[30], single voxel spectra were recorded from striatum in the monkeys using a standard point resolved spectroscopy (PRESS) sequence (TR/TE = 2000/272 ms and 2000/136 ms, 2-kHz sweep width) with presaturation of the water using three chemical shift selective suppression (CHESS) pulses. The voxels were prescribed from a coronal plane and were optimized to cover both caudate and putamen. The voxel sizes ranged from a minimum of $8 \times 8 \times 9$ mm^3 (0.6 cm^3) to a maximum of $1 \times 1 \times 1$ cm^3. Data were analyzed using the NMR1 (New Methods Research, Syracuse New York) software package. After apodization with an exponential multiplication corresponding to a 1–2-Hz line-broadening and Fourier transformation, the major metabolites[30] were integrated in the frequency domain using curve fitting and assuming mixed Lorenzian-Gaussian lineshapes. Metabolite intensities were normalized relative to the phosphocreatine/creatine peak at 3.03 ppm as the denominator.

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1. Gjaedde, A. & Wong, D.F. Modeling neuroreceptor binding of radioligands *in vivo*. *Quant. Imaging* 51–79 (1990).
2. Delforge, J. *et al.* Kinetic analysis of central (76)bromolisuride binding to dopamine D2-receptors studied by PET. *J. Cereb. Blood Flow Metab.* **11**, 914–925 (1991).
3. Leenders, K.L. Pathophysiology of movement disorders studied using PET. *J. Neural Transm. Suppl.* **50**, 39–46 (1997).
4. Cruz, C.J., Aminoff, M.J., Meyerhoff, D.J., Graham, S.H. & Weiner, M.W. Proton MR spectroscopic imaging of the striatum in Parkinson's disease. *Magn. Reson. Imaging* **15**, 619–624 (1997).
5. Holshouser, B.A. *et al.* Localized proton NMR spectroscopy in the striatum of patients with idiopathic Parkinson's disease: a multicenter pilot study. *Magn. Reson. Imaging* **33**, 589–594 (1995).
6. Brooks, D.J. PET studies on the early and differential diagnosis of Parkinson's disease. *Neurology* **43**, S6–S16 (1993).
7. Herholz, K. *et al. In vivo* imaging of glucose consumption and lactate concentration in human gliomas. *Ann. Neurol.* **31**, 319–327 (1992).
8. Alger, J.R., Frank, J.L., van Zijl, P.C., Moonen, C.T. & DiChiro, G. Metabolism of human gliomas: assessment with H-1 MR spectroscopy and F-18 fluorodeoxyglucose PET. *Radiology* **177**, 633–641 (1990).
9. Hugg, J.W. *et al.* Elevated lactate and alkalosis in chronic human brain infarction observed by 1H and 31P MR spectroscopic imaging. *J. Cereb. Blood Flow Metab.* **12**, 734–744 (1992).
10. Petroff, O.A. *et al.* Spectroscopic imaging of stroke in humans: histopathology correlates of spectral changes. *Neurology* **42**, 1349–1354 (1992).
11. Prichard, J. *et al.* Lactate rise detected by 1H NMR in human visual cortex during physiologic stimulation. *Proc. Natl. Acad. Sci. USA* **88**, 5829–5831 (1991).
12. Schapira, A.H. Pathogenesis of Parkinson's disease. *Baillieres Clin. Neurol.* **6**, 15–36 (1997).
13. Frost, J.J. *et al.* Positron emission tomographic imaging of the dopamine transporter with 11C-WIN 35,428 reveals marked declines in mild Parkinson's disease. *Ann. Neurol.* **34**, 423–431 (1993).
14. Ehringer, H. & Hornykiewicz, O. Distribution of noradrenaline and dopamine (3-hydroxytyramine) in the human brain and their behavior in diseases of the extrapyramidal system. *Klin. Wochenschr.* **38**, 1236–1239 (1960).
15. Dunnett, S.B., Whishaw, I.Q., Jones, G.H. & Isacson, O. Effects of dopamine-rich grafts on conditioned rotation in rats with unilateral 6-hydroxydopamine lesions. *Neurosci. Lett.* **68**, 127–135 (1986).
16. Ungerstedt, U. 6-Hydroxy-dopamine induced degeneration of central monoamine neurons. *Eur. J. Pharmacol.* **5**, 107–110 (1968).
17. Langston, J.W., Langston, E.B. & Irwin, I. MPTP-induced parkinsonism in human and non-human primates—clinical and experimental aspects. *Acta Neurol. Scand. Suppl.* **100**, 49–54 (1984).
18. Calne, D.D. *et al.* Positron emission tomography after MPTP. *Nature* **317**, 244–248 (1985).
19. Burns, R.S. *et al.* A primate model of parkinsonism: selective destruction of dopaminergic neurons in the pars compacta of the substantia nigra by n-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Proc. Natl. Acad. Sci. USA* **80**, 4546–4550 (1983).
20. Hantraye, P. *et al.* In vivo visualization by positron emission tomography of the progressive striatal dopamine receptor damage occuring in MPTP-intoxicated non-human primates. *Life Sci.* **39**, 1375–1382 (1986).
21. Imai, H., Nakamura, T., Endo, K. & Narabayashi, H. Hemiparkinsonism in monkeys after unilateral caudate nucleus infusion of 1-methyl-4-phenyl-1,2,3,6-tetrapyridine (MPTP): behavior and histology. *Brain Res.* **474**, 327–338 (1988).
22. Hantraye, P. *et al.* Dopamine fiber detection by [11 C]-CFT and PET in a primate model of parkinsonism. *Neuroreport* **3**, 265–268 (1992).
23. Wullner, U. *et al.* Dopamine terminal loss and onset of motor symptoms in MPTP-treated monkeys: a positron emission tomography study with 11C-CFT. *Exp. Neurol.* **126**, 305–309 (1994).
24. Temlett, J.A. Parkinson's disease: biology and aetiology. *Curr. Opin. Neurol.* **9**, 303–307 (1996).
25. Frohna, P.A., Rothblat, D.S., Joyce, J.N. & Schneider, J.S. Alterations in dopamine uptake sites and D1 and D2 receptors in cats symptomatic for and recovered from experimental parkinsonism. *Synapse* **19**, 46–55 (1995).
26. Strauss, I., Williamson, J.M., Bertram, E.H., Lothman, E.W. & Fernandez, E.J. Histological and 1H magnetic resonance spectroscopic imaging analysis of quinolinic acid-induced damage to the rat striatum. *Magn. Reson. Med.* **37**, 24–33 (1997).
27. Higuchi, T. *et al.* Effects of severe global ischemia on N-acetylaspartate and other metabolites in the rat brain. *Magn. Reson. Med.* **37**, 851–857 (1997).
28. Jenkins, B., G. *et al.* Non-invasive neurochemical analysis of focal excitotoxic lesions in models of neurodegenerative illness using spectroscopic imaging. *J. Cereb. Blood Flow Metab.* **16**, 450–461 (1996).
29. Guimareaeas, A.R. *et al.* Quantitative nuclear magnetic resonance spectroscopic imaging of neuronal loss in excitotoxic rat brain model. *Neuroscience* **69**, 1093–1101 (1995).
30. Arnold, D.L. *et al.* Use of proton magnetic resonance spectroscopy for monitoring disease progression in multiple sclerosis. *Ann. Neurol.* **36**, 76–82 (1994).
31. Davies, C.A. *et al.* Serial proton magnetic resonance spectroscopy in acute multiple sclerosis lesions. *Brain* **11**, 49–58 (1994).
32. Birken, D.L. & Olendorf, W.H. N-acetyl-L-aspartic acid: a literature review of a compound prominent in 1H-NMR spectroscopic studies of the brain. *Neurosci. Biobehav.* **1**, 23–31 (1989).
33. Urenjak, J., Williams, S.R., Gadian, D.G. & Noble, M. Proton nuclear magnetic resonance spectroscopy unambiguously identifies different neural cell types. *J. Neurosci.* **13**, 981–989 (1993).
34. Brownell, G.L. *et al.* Development in high-resolution positron emission tomography at MGH. *Int. J. Imag. Sys. Technol.* 1, 207–17 (1989).
35. Derenzo, S.E., Huesman, R.H. & Cahoon, J.L. A positron tomograph with 600 BGO crystals and 2.6 mm resolution. *IEEE Trans. Nucl. Sci.* **NS-35**, 659–664 (1988).
36. Brownell, G.L., Burnham, C.A. & Chesler, D.A. in *The Metabolism of the Human Brain Studied With Positron Emission Tomography,* Ed. (eds. Greitz, T., Ingvar, D.H. & Widen, L.) 13–19 (Raven, New York, 1985).
37. Bugiani, O., Perdelli, F., Salvarani, S., Leonardi, A. & Mancardi, G.L. Loss of striatal neurons in Parkinson's disease: a cytometric study. *Eur. Neurol.* **19**, 339–344 (1980).
38. Hwang, J.H. *et al.* Short echo time proton magnetic resonance spectroscopic imaging of macromolecule and metabolite signal intensities in human brain. *Magn. Reson. Med.* **35**, 633–639 (1996).
39. Frost, J.J. *et al.* Multicompartmental analysis of [11C]-carfentanil binding to opiate receptors in humans measured by positron emission tomography. *J. Cereb. Blood Flow Metab.* **9**, 398–409 (1989).
40. Kaufman, M.J. & Madras, B.K. Severe depletion of cocaine recognition sites associated with the dopamine transporter in parkinson's diseased striatum. *Synapse* **9** (1991).
41. McNeil, T.H., Brown, S.A., Rafols, J.A. & Shoulson, I. Atrophy of medium spiny I striatal dendrites in advanced Parkinson's disease. *Brain Res.* **455**, 148–152 (1988).
42. Federico, F. *et al.* Proton magnetic resonance spectroscopy in parkinson's disease and atypical parkinsonian disorders. *Mov. Disord.* **12**, 903–909 (1997).
43. Clarke, C.E., Lowry, M. & Horsman, A. Unchanged basal ganglia N-acetylaspartate and glutamate in idiopathic parkinson's disease measured by protonmagnetic resonance spectroscopy. *Mov. Disord.* **12**, 297–301 (1997).
44. Carvey, P. *et al.* Alterations in striatal neurotrophic activity induced by dopaminergic drugs. *Pharmacol. Biochem. Behav.* **46**, 195–204 (1993).
45. Brownell, A.-L. *et al.* Cocaine congeners as PET imaging probes for dopamine terminals. *J. Nucl. Med.* **37**, 1186–1192 (1996).
46. Chesler, D.A. Positron emission tomography and three-dimensional reconstruction technique. *Tomograph. Imag. Nuc. Med.* 176–183 (1973).
47. Foster, D.M. *et al.* SAAM II: simulation, analysis and modeling software. *BMES Bull* **18**, 19–21 (1994).

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Dossier: Parkinson's disease

Dopamine imaging markers and predictive mathematical models for progressive degeneration in Parkinson's disease

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Summary – We conducted PET imaging studies of modulation of dopamine transporter function and MRS studies of neurochemicals in idiopathic primate Parkinson's disease (PD) model induced by long-term, low-dose administration of MPTP. MR spectra showed striking similarities of the control spectrum of the primate and human striatum as well as MPTP-treated primate (six months after cessation of MPTP), and Parkinson's disease patient striatum (68 year old male; Hoehn-Yahr scale II; 510 mg/d L-DOPA). The choline/creatine ratio was similar in the MPTP model and human parkinsonism, suggesting a possible glial abnormality. The progressive degeneration of dopamine re-uptake sites observed in our PD model can be expressed by a time dependent exponential equation $N(t) = N_0 \exp(-(0.072 \pm 0.016)\, t)$, where N_0 represents intact entities (dopamine re-uptake sites before MPTP) and 0.072 per month is the rate of degeneration. When the signs of PD appear, N(t) is about 0.3–0.4 times N_0. Interestingly, this biological degenerative phenomena has similar progression to that observed in cell survival theory. According to this theory and calculated degeneration rate, predictive models can be produced for regeneration and protective treatments. © 1999 Elsevier, Paris

dopamine transporters / L-DOPA / MPTP / MRS / Parkinson's disease / PET

Parkinson's disease (PD) is one of the most common neurologic disorders. It is estimated that about 1 million Americans are affected by Parkinson's disease and about 40,000 new patients are diagnosed every year. Hypotheses of the etiology of PD are focused on possible genetic links (such as *α-synuclein*) and on the potential contribution of toxins (exogeneous and/or endogenous) [78, 79] and their potential interaction with genetic components [15]. At the cellular level PD is characterized by severe depletion of DA neurons and associated loss of synapses in the basal ganglia.

PD is diagnosed clinically based on the cardinal signs: tremor, rigidity, bradykinesia and postural instability [66]. Improved understanding of the pathophysiologic mechanism underlying parkinsonian signs and symptoms [70], as well as refinement of methods and techniques in neuroradiology, neurosurgery and neurophysiology, have stimulated the recent interest in developing therapeutic techniques. Investigations of MPTP (1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine)-induced parkinsonism in non-human primates have led to the hypothesis that dopamine deficiency in striatum leads to unbalanced activity from subthalamic nucleus into globus pallidus, resulting excessive inhibitory outflow (increased and synchronized spontaneous firing rate) from the internal segment of the globus pallidus [25]. This suppresses the motor thalamus which reduces activation of the cerebral cortex motor system, resulting in deficiency of movement [6, 25]. To interrupt this basal ganglia-motor system circuitry; three different therapeutic modalities are used, namely pharmacological therapy [52, 72, 80], fetal cell transplantation [28, 29, 51, 59], and surgical procedures such as pallidotomy [30, 36], thalamotomy [46] and chronic thalamic high frequency stimulation [4].

A recent extensive PD twin study indicates that physiological and toxic factors play roles in causing typical PD as humans age [79]. This progressive decline of dopamine (DA) terminals seen in idiopathic PD can be closely modeled in the non-human primate *Macaca fascicularis* by a low-dose exposure of the mitochondrial toxin, MPTP [8, 42, 81].

Developing radiopharmaceuticals for detection of dopamine terminals has been a major challenge for pharmacological research. Since autoradiographic studies of using cocaine analogs to label dopamine transporters were introduced [49], tropane derivatives have been widely used in PET imaging studies of

Parkinson's disease and drug abuse [35, 40, 52]. The latest developments, however, involve specific and sensitive cocaine analogs labeled with technetium-99m or iodine-123, used in single photon emission tomography studies of dopaminergic system [7, 22, 38, 53, 64].

The ability to observe both physiology and function in small areas within the brain is now possible with high resolution PET and MR imaging techniques [11, 16, 47]. The potential use of positron emission tomography (PET) as a research tool in movement disorders has been demonstrated in studies of brain dopamine function [74] and glucose metabolism associated with movement disorders [1, 43, 71]. Recently, high resolution PET imaging has been widely used in studies with animal models of Parkinson's disease [8-10, 18, 19, 42, 48, 81]. In addition, advances in receptor studies [10, 32, 42], and magnetic resonance spectroscopy of neurodegeneration [8, 24, 39, 44, 47], provide specific functional neurochemical information.

Our earlier work indicated; (1) that a stable Parkinson-like disease appears after chronic administration of a neurotoxin, MPTP; (2) that progressive dopaminergic fiber loss can be detected by positron emission tomography (PET) using carbon-11 labeled 2β-carbomethoxy-3β-(4-fluorophenyl) tropane (^{11}C-WIN 35,428 or ^{11}C-CFT) to label dopamine reuptake sites [42, 81]; and, (3) that progressive physiological changes of neurochemicals occur as observed with MRS and PET [8]. In the present article, we compare imaging characteristics of ^{11}C-CFT with those of ^{18}F-L-6-fluorodopa, and show that by using ^{11}C-CFT the progressive degeneration of dopamine terminals can be mathematically modeled to determine the rate of degeneration and predict the time of onset of PD signs.

MATERIALS AND METHODS

Study design

Longitudinal PET and MRS imaging studies were carried out in six MPTP-treated primates *(Macaca fascicularis)* to follow the progression of the MPTP-induced degeneration. These primates served as their own controls in studies prior MPTP. Control studies with MRS included four additional primates *(table I)*. Comparison of MRS primate data was done with one Parkinson's disease patient (68 year old male; Hoehn-Yarn scale II, 510 mg/d L-DOPA) and an aged matched normal volunteer.

MPTP-lesion in primates

A slow neurotoxic lesion of dopaminergic cells located in the substantia nigra and in the ventral tegmental area was obtained by repetitive administration of MPTP dissolved in saline and immediately administered intravenously to primates (0.6 mg/kg i.v., every two weeks until behavioral stability) under light anesthesia (ketamine, 5 mg/kg i.m.), as previously described [81].

Table I. Striatal neurochemical changes in primates 0.5–2 years after cessation of MPTP treatment.

Metabolite Ratio	*Controls (n = 10)*	*MPTP (n = 6)*
NAA/Cr (range)	2.38 ± 0.11 (2.3–2.5)	2.09 ± 0.29* (1.7–2.5)
Cho/Cr (range)	0.83 ± 0.06 (0.8–0.9)	1.20 ± 0.15*** (1.0–1.4)

Unpaired Student's t test values for difference from control: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

In this chronic model, behavioral signs developed gradually over 9–14 months, progressing from bradykinesia to akinesia in all limbs. Tremor also occurred as the last PD sign. These signs did not spontaneously recove, in contrast to acutely induced MPTP-PD models [20, 31, 54].

PET imaging studies of dopamine transporters

Instrumentation

Positron emission tomography studies were carried out with PET scanning system, PCR-I [11], as earlier described [8].

Labeling of radiopharmaceuticals

Radiolabeling of ^{11}C-CFT was published earlier [10] and L-6-^{18}F-fluorodopa was prepared according to the fluorodemercuration method [62].

Experimental procedures

For PET imaging, animals were anaesthetized with ketamine/xylazine (30/3 mg/kg i.m.) initial dose and anesthesia was maintained with half a dose hourly injections as needed. Catheterization of the femoral artery and vein was used for collection of blood samples and injection of labeled ligand. The animal was placed in the imaging position, and the head was adjusted into a stereotactic headholder with the earbar at the origin. Interior orbital supports ensure that images were acquired in pseudocoronal plane perpendicular to the orbito-meatal line. This allows superposition of data from MRI and MRS studies. After the injection of labeled ligand, ^{11}C-CFT or ^{18}F-L-6-fluorodopa (5mCi, specific activity 600–1,000 mCi/μmol) into the femoral vein, imaging data were collected stepwise on seven levels (A30 (30 mm anterior from the origin), A25, A20, A15, A10, P5 (5 mm posterior from the origin) and P10) initially using 15 s frames. The frame time was subsequently increased to

60 s, the total imaging time being 90 min for ^{11}C-CFT and 120 min for ^{18}F-L-6-fluorodopa. While imaging with ^{11}C-CFT, 18 arterial blood samples of 0.1 mL were collected at different time points starting from 10 s frequency and ending with 15 min frequency to monitor the decrease in radioactivity. In addition three arterial blood samples were collected for HPLC analyses of metabolites of labeled ligand.

Calibration of the positron tomograph was performed in each study session using a cylindrical plastic phantom (diameter 6 cm) and ^{18}F-solution. Cross calibration with a gamma counter (Packard Cobra Auto-gamma, Downers Grove, IL, USA) was carried out using the same solution. Imaging data were corrected for uniformity, sensitivity, attenuation, decay and collection time. PET images were reconstructed using Hanning weighted convolution back-projection [13]. Regions of interest including left and right caudate and putamen, frontal, parietal and temporal cortex, thalamus and cerebellum were drawn on each level and activity per unit volume, percent activity of injected dose, and ligand concentration were calculated. Plasma data were corrected for counting efficiency, calibration factor and measured metabolites of ^{11}C-CFT and percent activity of injected dose and ligand concentration were calculated. Plasma data was used as an input function in the kinetic modeling.

Receptor studies with ^{11}C-labeled CFT

The kinetic behavior of ^{11}C-CFT was studied with a three compartmental model approach [77]. In the three compartmental model, the first compartment is the plasma pool, the second is the exchangeable tracer pool including free and nonspecifically bound ligand in the brain, and the third compartment is a trapped tracer pool including bound ligand in the brain. The exchangeable tracer pool contains ligand but no receptors and the third compartment includes all the receptors, partly or totally occupied by ligands. The kinetic parameters k_3 and k_4 describe the binding to and dissociation from receptors.

The transfer coefficients k_1–k_4 were mathematically resolved using the SAAM II program [26]. For stabilization of the k values the fitting procedure was performed using three steps. Since cerebellum does not have specific receptor binding or it is negligible, fitting was done in the cerebellum data letting all the k-values float. Briefly, with estimates for the initial conditions for the k-values, the differential equations were integrated using an adaptable fourth order Runge-Kutta method with suitable accuracy (tolerance 1^{e-7}). Iterations continued until sufficient convergence was achieved for the system parameters (k_1–k_4). The ratio k_1/k_2 was calculated. In further iterations of the striatal data the fixed ratio (k_1/k_2) was used as a constraint to reach parameter optimization. Regional binding parameters k_3/k_4 were calculated for each study.

Comparison of imaging characteristics of ^{11}C-CFT and ^{18}F-L-6-fluorodopa

Comparison of imaging characteristics of ^{11}C-CFT and ^{18}F-L-6-fluorodopa was based on obtained contrast in striatum compared to cerebellum. The difference of the striatal and cerebral accumulation of radioactivity was fitted into gamma variate function and the maximum value was divided by the value of the cerebral activity at that time point.

Modeling of progressive degeneration

To analyze MPTP-induced progressive degeneration, values of striatal binding potentials of ^{11}C-CFT at different time points during the MPTP-administrations (time = 0 when MPTP-administration was started) were fitted into an exponential function; $N(t) = N_0(t = 0) \exp(-k\,t)$. N_0 denotes binding potential in the intact dopamine terminals or arbitrary estimate of the intact dopamine terminals, N(t) is the corresponding value after degeneration of time (t) and k is a rate of degeneration.

MRS studies of neurochemicals

For these studies, we utilized single voxel spectroscopy of the basal ganglia. We chose voxels centered in the striatum for both monkeys and PD patients. Voxels were between 0.5–1 cm^3 in the monkey brain and between 3–5 cm^3 in the human brain. Water suppression was performed using CHESS pulses and localization by a standard PRESS-type sequence with TR/TE of either 2000/272 or 2000/136 ms. Spectra were processed using the NMR1 program (NMRI, Syracuse, NY), by curve fitting the entire spectrum and integrating the areas of the major metabolites. Integrals were then normalized to the creatine peak at 3.03 ppm (Cr) as a standard.

Metabolite quantification

We found NAA/Cr ratios to be reliable quantitative indicators of neurodegeneration. This reliability was indicated by the large differences noted between the MPTP-lesioned animals. In the case of single voxel spectroscopy we used a fully relaxed non-water suppressed spectrum from the voxel. This provides a constant internal reference for a metabolite/water ratio even if, due to metabolite T1 and T2 errors, absolute concentrations remain elusive. The stan-

dard deviations in this technique were very small, and allowed to make direct inter-animal comparisons.

Characterization of the elevated lipid/lactate peaks were performed using multiple TE values to characterize the coupling constants and double quantum filtration to estimate how much of the intensity is due to lactate. Due to the relatively shorter T1 values of lipids, we used inversion recovery PRESS spectra with variable TI values to characterize the lipid T1s in order to estimate the concentrations. In addition, we have implemented a STEAM sequence with the capability getting TE's down to 6 ms. This enables quantitative measurements of the lipid and macromolecular components when combined with the inversion recovery experiments.

RESULTS

Figure 1 shows ^{11}C-CFT and ^{18}F-L-6-fluorodopa distribution in the same control primate. Sixty minutes before the ^{18}F-L-6-fluorodopa injection the primate was pretreated with carbidopa (5 mg/kg) to reduce peripheral metabolism. These images show the striking specificity of ^{11}C-CFT to image striatal function. The contrast of striatal binding using ^{11}C-CFT was 3.25 ± 0.56 and correspondingly 1.67 ± 0.23 using ^{18}F-L-6-fluorodopa. Striatal data were averaged from putamen data of levels A20 and A15 from the left and right sides and caudate data of levels A25 and A20 from both sides. *Figure 2* shows relative ^{11}C-CFT binding distribution before and during MPTP administration in an asymptomatic and symptomatic stage. Three coronal brain levels (A25, A20 and A15) through the striatum show that degeneration in putamen is more severe that in caudate. The progressive degeneration of dopamine reuptake sites observed in our primate PD model can be expressed by an exponential equation $N(t) = N_0 \exp(-k\,t)$, where N_0 represents intact entities (dopamine reuptake sites) and k represents the rate of progressive degeneration. *Figure 3* shows progressive degeneration observed in six primates during low-dose MPTP administrations. The exponential curve fitted to the calculated binding potential values is $N(t) = N_0 \exp(-(0.072 \pm 0.016)\,t)$ indicating that the rate of MPTP-induced degeneration is 0.072 per month. When signs of PD appeared, N(t) was about (0.3–0.4) N_0.

We have also investigated neurochemical changes with MRS in the same primates as imaged by PET using ^{11}C-CFT. Spectra from a control and typical MPTP-treated primate striatum (six months after cessation of MPTP therapy) is shown in *figure 4* with comparison to MR spectra of a parkinsonian patient (68 year old male, Hoehn-Yahr scale II, 510 mg/d L-DOPA) and an age matched control patient. Note the pronounced changes

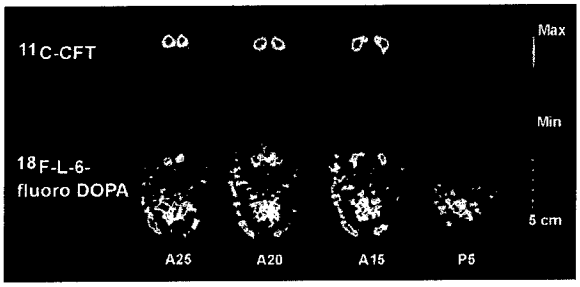

Figure 1. Color coded PET images showing ^{11}C-CFT and ^{18}F-L-6-fluorodopa accumulation in the same control primate brain. Sixty minutes before fluorodopa injection the animal was pretreated with carbidopa (5 mg/kg) to reduce peripheral dopamine metabolism. ^{11}C-CFT images are acquired 60–62 min after injection and ^{18}F-L-6-fluorodopa images 90–120 min after injection. Four images represent the brain levels A25, A20, A15 mm anterior and P5 mm posterior from the reference plain. After corrections for decay, acquisition time and injected activity the highest pixel value of the four ^{11}C-CFT images was normalized to 10,000 and the lowest to 0. All the ^{11}C-CFT images were normalized according to this scale. Correspondingly, after corrections the four ^{18}F-L-6-fluorodopa images were normalized similarly.

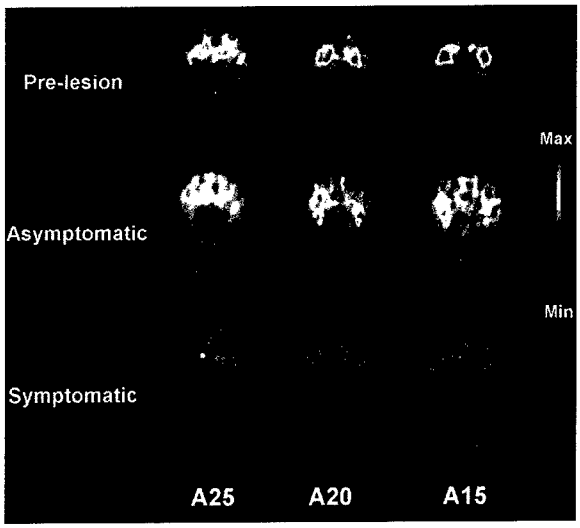

Figure 2. Color coded PET images showing relative ^{11}C-CFT binding in a monkey brain 60–62 min after injection. The three images represent the levels throughout caudate-putamen (A25, A20 A15 mm anterior of the reference plain) before MPTP treatment, after 3 MPTP injections, when the primate was asymptomatic and after 9 months of MPTP treatment, when the monkey was symptomatic. After corrections for decay, acquisition time and injected activity, the average count density was determined in cerebellum study and ^{11}C-CFT images of the three coronal brain levels were divided by this value on the pixel basis individually in each study. Finally, the highest pixel value in the nine images was normalized to 10, 000 and the lowest to 0. All the images were normalized according to this scale.

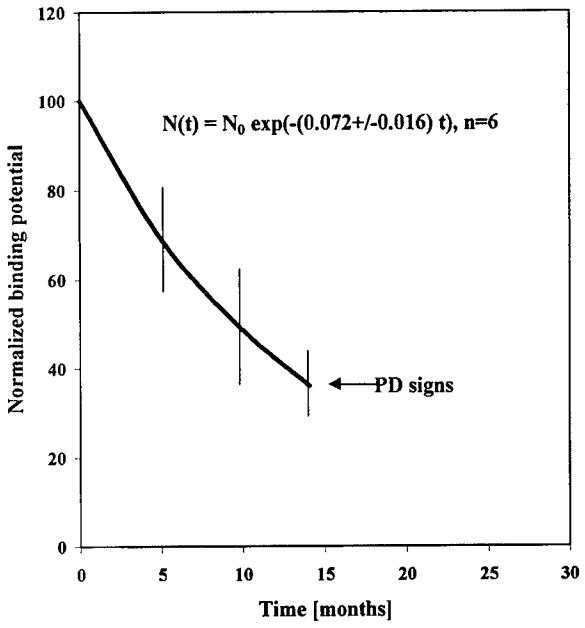

Figure 3. Model for the progressive degeneration and the appearance of parkinsonism in MPTP treated primates. Control value of the binding potential (before MPTP) was normalized individually to 100 and all the other values were normalized according this scale. (Raw data from [8].)

compared to the control striatum. Lactate and/or lipid peaks are visible in both the patient and the primate, but not in the controls. In all the primates studied (n = 6), the lactate/lipid peaks had disappeared after an additional eight months [8]. These data indicate an acute metabolic process which resolves after a period of time, and is consistent with the time course for macrophage infiltration. Unfortunately we were unable to collect enough data to completely assay the time course of changes in all the metabolites over time. Future studies will entail collection of more data to determine the complete spectroscopic time profile of evolution of the neurochemical changes.

In the MPTP model there is a significant decrease in NAA, which is larger than that seen in our PD patients (NAA/Cr = 2.09, n = 6 vs. 2.33 in PD patients, n = 23, B. Jenkins, personal communication). This is significant since our control human population had identical NAA/Cr levels to the primate controls (2.33 ± 0.46 in humans; n = 20 vs. 2.38 ± 0.11 in primates, n = 10, Jenkins, personal communication). Notably, in the MPTP monkeys there was a large increase in the Cho/Cr ratio, very similar to what is seen in our PD patients (Cho/Cr = 1.2). Choline may be reflective of gliosis as the choline concentration in glial cells is twice that in neurons or of macrophage activity. A quantitative summary of our primate results is shown in the *table I*.

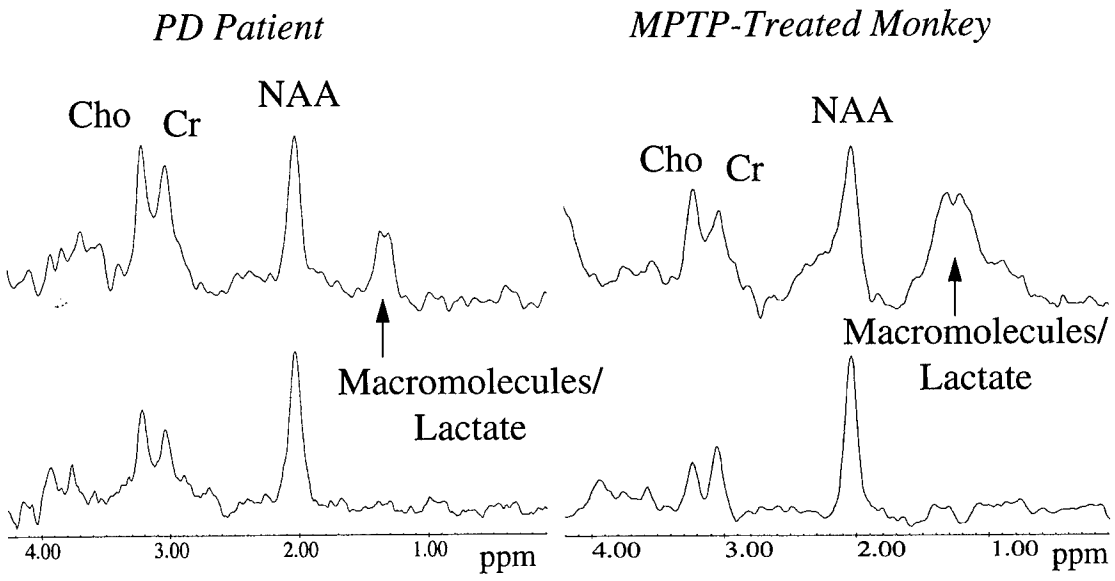

Figure 4. Striatal spectra from: Left) A PD patient (male; 68 years old; Hoehn-Yahr scale II; 510 mg/d L-DOPA), and an age-matched control. Right) An MPTP-treated monkey 6 months after cessation of MPTP-treatment and a control monkey. Major neurochemicals observed are indicated. Note the striking similarity of the control spectrum of the primate and human as well MPTP-treated primate and Parkinson's disease patient (TR/TE 2000/272ms; PRESS).

DISCUSSION

Realistic primate models that mimic the progressive changes of PD are of critical importance for developing neural therapeutic techniques. The optimal procedure for therapy-induced behavioral recovery observed in many clinical and experimental studies is still unclear.

Primate models of parkinsonism were developed using MPTP administered according to different protocols [12, 56]. Stereotaxic application of MPTP (or its active metabolite MPP+) in substantia nigra or in the striatum, as well as intra-carotid injections or repeated intravenous administration during 5–10 days [12, 45, 56], generally induces a marked dopamine depletion resulting in a severe akineto-rigid parkinsonian syndrome (often requiring drug therapy) within weeks following treatment. Such studies demonstrated that MPTP-induced behavioral, neurochemical and anatomical changes are analogous but not identical to alterations observed in parkinsonian patients [12, 21]. Acute protocols (toxicity induced over one to five days) of MPTP differ from idiopathic (PD) in several aspects: (1) pathologic changes in idiopathic PD extend beyond the substantia nigra [37]; whereas, the substantia nigra, and to a lesser extent the ventral tegmental area, are the regions primarily lesioned by MPTP toxicity; (2) acute MPTP-administration to non-human primates does not produce an uneven pattern of striatal dopamine loss described in idiopathic PD, with relative sparing of dopamine levels in the caudate nucleus compared to the putamen [21]; (3) acute MPTP toxicity in non-human primates also creates motor symptoms that may recover with time [20, 54]; (4) an acute administration protocol does not reproduce the chronic and slow degeneration of dopamine neurons that occurs in idiopathic PD. Recently, a less acute primate model of various stages of PD has been obtained by unilateral intra carotid infusion [48] combined with sequential systemic doses of MPTP [19]. In addition, a chronic model of PD has been introduced by using daily low dose systemic injections of MPTP for 22 days [5].

Following these principles, our studies involving chronic low-dose administration of MPTP [8], have clearly demonstrated that by repeated administration of the neurotoxin over a long period of time, it is possible to increase the selectivity of the neurotoxin for specific subpopulations of dopamine neurons, more accurately reproducing the pattern of neuropathological and neurochemical alterations observed in idiopathic PD.

Recent advances of in vivo receptor studies have resulted in the development of new receptor specific ligands [2, 23, 32, 33, 63] combined with advances in instrumentation for PET [3, 11, 16]. High resolution positron imaging yields accurate data over small regions inside the brain [9] that, combined with modeling of the ligand-receptor interaction, can provide valuable quantitative information about receptor behavior in different areas of the living brain.

Modeling of neuroreceptor kinetics has also been an active research area. Several methods have been proposed for estimating the binding parameters (B_{max}, maximum available receptor binding sites; K_D, dissociation constant; k_{on}, bimolecular association rate constant; and, k_{off}, dissociation rate). The choice of method depends on the particular properties of ligand-receptor interaction. In reversible binding, ligands dissociate from the receptor during the imaging period so that the maximum binding site density can be calculated from the equilibrium distribution [23]. In the case of irreversible binding, equilibrium is not achieved during the imaging period. The dopamine transporter specific ligand (^{11}C-CFT) has irreversible binding.

Two types of kinetic analysis are used to analyze PET data. The graphical method [60, 73] has been applied by our group to estimate the influx of ^{11}C-CFT to dopamine terminals [42], and by several groups in estimating the influx of L-6-^{18}F-fluorodopa [59, 67].The other method is based on general non-linear regression techniques [14, 61, 69, 77].

Research has demonstrated a significant correlation between depression of striatal ^{18}F-L-fluorodopa uptake of PD patients and their degree of locomotor disability. However, while the average putamen ^{18}F-L-dopa uptake in PD is reduced to 40% of normal, a 60% loss of nigra compacta cells and 80–90% loss of putaminal dopamine levels are found post-mortem in PD [34]. Therefore, striatal ^{18}F-L-fluorodopa uptake reflects metabolic and functional activity of nigro-striatal fibers, but may not accurately depict levels of endogenous striatal dopamine or anatomical depletion of dopamine terminals. A specific tracer for selective labeling of dopamine fibers would be preferable. Among various candidates for labeling dopaminergic fibers, specific ligands for dopamine re-uptake sites (dopamine transporter) such as ^{11}C-nomifensine, ^{11}C-cocaine or ^{18}F-GBR 13119 (1–((4–((18F)fluorophenyl) (phenyl)methoxy) ethyl)-4-(3-phenylpropyl) piperazine) have been used in PET studies [27, 50, 57]. In such PET studies, specific binding of the ligands to dopamine transporters were taken as a measure of monoaminergic nerve terminal density. However, using these ligands in vitro, binding assay showed only a 40% decrease of binding in the caudate nucleus and putamen of subjects with PD [65], while other measures for

dopaminergic terminals were reduced much more dramatically. Similar results have been obtained in vivo using ^{11}C-S-nomifensine as a PET tracer [58]. Again, the 40% decrease in dopamine re-uptake site density is strikingly different from the 90% decrease of dopamine levels measured post-mortem in parkinsonian putamen.

We have studied the imaging characteristics of carbon-11 labeled CFT in normal and MPTP-treated primates [10], and it has proved to be a very selective ligand [68] to monitor dopamine terminal degeneration having higher specificity than nomifensine or GBR analogues for the dopamine uptake complex [49]. Several observations suggest that CFT is a useful and specific marker for dopamine nerve terminal density: ^{11}C-CFT in vivo binding, as well as ^{3}H-CFT in vitro binding [49] in the non-human primate caudate nucleus, is highly specific for the dopamine transporter. ^{3}H-CFT binding was decreased in PD up to 95% depending on striatal region [49], and ^{3}H-CFT depletion in PD paralleled the dopamine depletion, with a more severe decrease in specific binding in the putamen than in the caudate nucleus [49].

Our group was the first to demonstrate that ^{11}C-CFT binding correlated with behavioral symptoms in a primate model of Parkinson's disease [42]. This has been verified in a larger series of primates [81], and also in early Parkinson's disease in humans [32]. After the earlier studies, several novel tropane derivatives have been introduced for imaging of dopamine transporters, mainly labeled with iodine-123 (altropane [64], beta-CIT [22], FP-CIT [7], PE21 [38] or technetium-99m (trodat) [53].

Figure 1 shows that the radiolabeled cocaine analog ligands e.g., ^{11}C-CFT provide better sensitivity and selectivity for imaging of the striatal dopamine system than radiolabeled L-dopa. *Figure 1* also demonstrates the effect of the increased active radiolabeled metabolites during imaging with ^{18}F-L-6-fluorodopa in blood rich areas in the head. ^{11}C-CFT used in PET imaging of MPTP treated monkeys demonstrate progressive DA terminal loss in caudate-putamen before and after appearance of PD signs. In addition, the observed MPTP-induced degeneration is more progressive in putamen than in caudate *(figure 2)*. Our new MRS studies illustrate lactate/lipid elevation in the striatum in both parkinsonian monkeys (post-MPTP) and in a typical case of a Parkinson's disease patient (68 year old male, Hoehn-Yahr scale II). This is consistent with previous studies [8], showing parallel increases in striatal lactate/lipid and continuous DA fiber (^{11}C-CFT) degeneration. In addition, the small decrease in NAA (12%) observed in the monkeys may also be reflective of the loss of dopamine terminals and striatal cell dendritic density.

Notably in the MPTP monkeys, there was a large increase in the Cho/Cr ratio which was almost identical to that of PD patients (Cho/Cr = 1.2). This is possibly an important physiological observation, since choline may reflect gliosis or magrophage activity. The various theories for neurodegeneration in PD includes one of loss of target-derived trophic support [17, 75, 76]. Glial cells typically provide both growth-factors and homeostatic support [75, 76, 82]. This finding deserves further investigation to determine if sub glial changes are a consequence or a primary cause of dopaminergic axonal degeneration in the caudate-putamen of PD.

Our data provides a basis for a mathematical model of degeneration of the DA system in PD. It is known that 60–70% degeneration in a dopaminergic system precedes the symptoms of PD. In our primate PD model, the remaining entities (dopamine re-uptake sites) were (0.3–0.4) of the original value when the PD signs appeared. Interestingly, this biological degenerative phenomena has similar progression to that formulated in cell survival theory in radiobiology concerning the effect of radiation in killing cells [41]. According to the formula, the number of survived cells (N_D) after radiation dose (D) is $N_D = N_0 \exp(-D/D_0)$, where N_0 is the number of cells before radiation and D_0 is the mean lethal dose of radiation. When the radiation dose (D) equals to the mean lethal dose (D_0), the function will get a form of $N_D/N_o = e^{-1} = 0.37$ and the number of survived cells is $0.37\ N_0$. Similarly, using the rate of degeneration (0.072 ± 0.016, *figure 3*), the calculated time to get PD signs is 13.9 ± 2.5 months in this MPTP-PD model, which is the same as was observed in experimental studies *(figure 3)*. With this theory and imaging studies of the dopaminergic system, a realistic estimate can be obtained of degeneration rate and the time when the patient will get PD symptoms.

CONCLUSION

^{11}C-CFT is a useful ligand for detection of PD-like progressive degeneration. Based on the decrease of ^{11}C-CFT binding, a rate of degeneration can be calculated and the time of onset of PD symptoms can be determined.

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REFERENCES

1 Antonini A, Leenders KL, Vontobel P, Maguire RP, Missimer J, Psylla M, et al. Complementary PET studies of striatal neuronal function in the different diagnosis between multiple system atrophy and Parkinson's disease. Brain 1997 ; 120 : 2187.

2 Arnett CD, Fowler JS, Wolf AP, Shiue CY, McPherson DW. ^{18}F-Methylspiroperidol: the radioligand of choice for PETT studies of the dopamine receptor in human brain. Life Sci 1985 ; 36 : 1359.

3 Bailey DL, Young H, Bloomfield PM, Meikle SR, Glass D, Myers MJ, et al. ART – a continuously rotating PET camera: performance characteristics, initial studies, and installation considerations in a nuclear medicine department. Eur J Nucl Med 1997 ; 24 : 6.

4 Benabid AL, Pollak P, Gao D, Hoffman D, Limousin P, Gay E. Chronic electrical stimulation of the ventralis intermedius nucleus of the thalamus as a treatment of movement disorders. J Neurosurg 1996 ; 84 : 203.

5 Bezard E, Imbert C, Deloire X, Bioulac B, Gross CE. A chronic MPTP model reproducing the slow evolution of Parkinson's disease: evolution of motor symptoms in the monkey. Brain Rese 1997 ; 766 : 107.

6 Bergman H, Wichmann T, Karmon B, DeLong MR. The primate subthalamic nucleus. II. Neuronal activity in the MPTP model of parkinsonism. J Neurophys 1994 ; 72 : 507.

7 Booij J, Busemann Sokole E, Stabin MG, Janssen AGM, de Bruin K, van Royen AE. Human biodistribution and dosimetry of (123I)FP-CIT: a potent radioligand for imaging of dopamine transporters. Eur J Nucl Med 1998 ; 25 : 24.

8 Brownell AL, Jenkins BG, Elmaleh DR, Deacon TW, Spealman RD, Isacson O. Combined PET/MRS brain studies show dynamic and long-term physiological changes in a primate model of Parkinson disease. Nature Med 1998 ; 4 : 1308.

9 Brownell AL, Livni E, Galpern W, Isacson O. PET imaging of dopamine terminals in a rat transplant model of Parkinson disease. Ann Neurol 1998 ; 43 : 387.

10 Brownell AL, Elmaleh DR, Meltzer P, Shoup TM, Brownell GL, Fischman AJ, et al. Cocaine congeners as PET imaging probes for dopamine terminals in normal and MPTP induced parkinsonism in nonhuman primate brain. J Nucl Med 1996 ; 37 : 1186.

11 Brownell GL, Burnham CA, Stearns CW, Chesler DA, Brownell AL, Palmer M. Development in high-resolution positron emission tomography at MGH. Int J Imig Systems and Technol 1989 ; 1 : 207.

12 Burns RS, Chiueh CC, Markey SP, Ebert MH, Jacobowitz DM, Kopin IJ. A primate model of parkinsonism : selective destruction of dopaminergic neurons in the pars compacta of the substantia nigra by N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. Proc Natl Acad Sci USA 1983 ; 80 : 4546.

13 Chesler DA. In: Freedman GS, ed. Tomographic Imaging in Nuclear Medicine. New York: The Society of Nuclear Medicine; 1973. p. 176.

14 Delforges J, Loc'h C, Hantraye P, Stulzaft O, Khalili-Varasteh M, Maziere M, et al. Kinetic analysis of central (76Br) bromolisuride binding to dopamine D2-receptor studies by PET. J Cer Blood Flow and Metab 1991 ; 11 : 914.

15 De Michele G, Filla A, Volpe G, Goglieltino A, Ambrosio G, Campanella G, et al. Etiology of Parkinson's disease: the role of environment and heredity. In: Battistin L, et al., eds. Adv Neurol. Philadelphia: Lippincott-Raven; 1996. p. 16.

16 Derenzo SE, Hueasman RH, Cahoon JL. A positron tomograph with 600 BGO crystals and 2.6 mm resolution. IEEE Trans Nucl Sci 1988 ; 35 : 659.

17 di Porzio U, Daguet MC, Glowinski J, Prochiantz A. Effect of striatal cells on in vitro maturation of mesencephalic dopaminergic neurons grown in serum-free conditions. Nature 1980 ; 288 : 370.

18 Doudet DJ, Chan GL, Holden JE, McGeer EG, Aigner TA, Wyatt RJ, et al. 6-[18F]Fluoro-L-DOPPA PET studies of the turnover of dopamine in MPTP-induced parkinsonism in monkeys. Synapse 1998 ; 29 : 225.

19 Eberling JL, Jagust WJ, Taylor S, Bringas J, Pivirotto P, VanBrocklin HF, et al. A novel MPTP primate model of Parkinson's disease: neurochemical and clinical changes. Brain Res 1998 ; 805 : 259.

20 Eidelberg E, Brooks BA, Morgan WW, Walden JG, Kokemoor RH. Variability and functional recovery in the N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine model of parkinsonism in monkeys. Neuroscience 1986 ; 18 : 817.

21 Elsworth JD, Deutch AY, Redmond DEJ, Taylor JR, Sladek JR, Ruth RH. Symptomatic and asymptomatic 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine treated primates: biochemical changes in striatal regions. Neuroscience 1989 ; 33 : 323.

22 Emond P, Farde L, Chalon S, Belzung C, Mauclaire V, Chiron JP, et al. Toxicity, mutagenicity, and behavioral effects of beta-CIT, a ligand for dopamine transporter exploration by SPECT. Nucl Med Biol 1998 ; 25 : 405.

23 Farde L, Hall H, Ehrin E, Sedvall G. Quantitative analysis of D2 dopamine receptor binding in the living human brain by PET. Science 1986 ; 231 : 258.

24 Federico F, Simone IL, Lucivero V, Iliceto G, De Mari M, Giannini P, et al. Proton magnetic resonance spectroscopy in Parkinson's disease and atypical parkinsonian disorders. Mov Disord 1997 ; 12 : 903.

25 Filion M, Tremblay L. Abnormal spontaneous activity of globus pallidus neurons in monkeys with MPTP-induced parkinsonism. Brain Res 1991 ; 547 : 142.

26 Foster DM. SAAM II: simulation, analysis and modeling software. BMES Bull 1994 ; 18 : 19.

27 Fowler JS, Volkow ND, Wolf AP, Dewey SL, Schlyer DJ, MacGregor RR, et al. Mapping cocaine binding sites in human and baboon in vivo. Synapse 1989 ; 4 : 371.

28 Freed CR, Breezr RE, Rosenberg NL, Schneck SA, Kriek E, Qi JX, et al. Survival of implanted fetal dopamine cells and neurologic improvement 12 and 46 months after transplantation for Parkinson's disease. N Engl J Med 1992 ; 327 : 1549.

29 Freeman TB, Olanow CW, Hauser RA, Nauert M, Smith DA, Borlongan CV, et al. Bilateral fetal nigral transplantation into the postcommissural putamen in Parkinson's disease. Ann Neurol 1995 ; 38 : 379.

30 Friedman JH, Epstein M, Sanes JN, Lieberman P, Cullen K, Lindquist C, et al. Gamma knife pallidotomy in advanced Parkinson's disease. Ann Neurol 1996 ; 39 : 535.

31 Frohna PA, Rothblat DS, Joyce JN, Schneider JS. Alterations in dopamine uptake sites and D1 and D2 receptors in cats symptomatic for and recovered from experimental parkinsonism. Synapse 1995 ; 19 : 46.

32 Frost JJ, Rosier AJ, Reich SG, Smith JS, Ehlers MD, Snyder SH, et al. Positron emission tomographic imaging of the dopamine transporter with ^{11}C-WIN 35,428 reveals marked decline in mild Parkinson's disease. Ann Neurol 1993 ; 34 : 423.

33 Garnett ES, Firnau G, Nahmias C. Dopamine visualized in the basal ganglia of living man. Nature 1983 ; 305 : 137.

34 German DC, Manaye K, Smith WK, Woodwart DJ, Saper CB. Midbrain dopaminergic cell loss in Parkinson's disease: Computer visualization. Ann Neurol 1989 ; 26 : 507.
35 Ginovart N, Lundin A, Farde L, Halldin C, Backman L, Swahn CG, et al. PET study of the pre- and post-synaptic dopaminergic markers for the neurodegenerative process in Huntington's disease. Brain 1997 ; 120 : 503.
36 Grafton ST, Waters C, Sutton JFLM, Couldwell W. Pallidotomy increases activity of motor association cortex in parkinson's disease: a positron emission tomographic study. Ann Neurol 1995 ; 37 : 776.
37 Graybiel AM, Hirsch E, Agid Y. The nigrostriatal system in Parkinson's disease. In: Advances in Neurology. New York: Raven Press; 1990. p. 17.
38 Guilloteau D, Emond P, Baulieu JL, Garreau L, Frangin Y, Poucelot L, et al. Exploration of the dopamine transporter: in vitro and in vivo characterization of a high-affinity and high-specificity iodinated tropane derivative (E)-N-(3-iodoprop-2-enyl)-2beta-carbomethoxy-3beta-(4'-methylphenyl) notropane (PE21). Nucl Med Biol 1998 ; 25 : 331.
39 Guimareas AR, Schwartz P, Prakash MR, Carr CA, Berger UV, Jenkins BG, et al. Quantitative nuclear magnetic resonance spectroscopic imaging of neuronal loss in excitotoxic rat brain model. Neuroscience 1995 ; 69 : 1095.
40 Gunn RN, Lammertsma AA, Hume SP, Cunningham VJ. Parametric imaging of ligand-recptor binding in PET using a simplified reference region model. Neuroimage 1997 ; 6 : 297.
41 Hall EJ. In: Radiobiology for the Radiologist. Philadelphia: J.B. Lippincott Co; 1994.
42 Hantraye P, Brownell AL, Elmaleh DR, Spealman RD, Wullner U, Brownell GL, et al. Dopamine fiber detection by (11C)-CFT and PET in a primate model of parkinsonism. Neuro Report 1992 ; 3 : 265.
43 Hirato M, Ohye C, Takahashi A, Negishi M, Shibasaki T. Study on the function of basal ganglia and frontal cortex using depth microrecording and PET scan in relation to the outcome of pallidotomy for the treatment of rigid-akinesia-type Parkinson's disease. Stereotact Funct Neurosurg 1997 ; 69 : 86.
44 Holshouser BA, Komu M, Moller HE, Zijlmans J, Kolem H, Hinshaw DB Jr, et al. Localized proton NMR spectroscopy in the striatum of patients with idiopathic Parkinson's disease: a Multicenter pilot study. Magn Reson Med 1995 ; 33 : 589.
45 Imai H, Nakamura T, Endo K, Narabayashi H. Hemiparkinsonism in monkeys after unilateral caudate nucleus infusion of 1-methy-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP): Behavior and histology. Brain Res 1988 ; 474 : 327.
46 Jankovic J. Treatment of parkinsonian syndromes. In: Kurlan R, ed. Treatment of movement disorders. Philadelphia: Lippincott; 1995. p. 95.
47 Jenkins BG, Brouillet E, Storey E, Chen YI, Schultz JB, Kirschner P, et al. Non-invasive neurochemical analysis of focal excitotoxic lesions in models of neurodegenerative illness using spectroscopic imaging. J Cereb Blood Flow Metab 1996 ; 16 : 450.
48 Jordan S, Eberling JL, Bankiewicz KS, Rosenberg D, Coxson PG. 6-(18F)fluoro-L-m-tyrosine: metabolism, positron emission tomography kinetics, and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine lesions in primates. Brain Res 1997 ; 750 : 264.
49 Kaufman MJ, Madras BK. Severe depletion of cocaine recognition sites associated with the dopamine transporter in Parkinson's diseased striatum. Synapse 1991 ; 9 : 43.
50 Kilbourn MR. In vivo binding of (18F)GBR 13119 to the brain dopamine uptake system. Life Sci 1988 ; 42 : 1347.
51 Kordower JH, Freeman TB, Snow BJ, Vingerhoets FJG, Mufson EJ, Sanberg PR, et al. Neuropathological evidence of graft survival and striatal reinnervation after the transplantation of fetal mesencephalic tissue in a patient with Parkinson's disease. N Engl J Med 1995 ; 332 : 1118.
52 Kotian P, Abraham P, Lewin AH, Mascarella SW, Boja JW, Kuhar MJ, et al. Synthesis and ligand binding study of 3 beta-(-substituted phenyl)-2 beta-(heterocyclic) tropanes. J Med Chem 1995 ; 38 : 3451.
53 Kung MP, Stevenson DA, Plossl K, Meegalla SK, Beckwith A, Essman WD, et al. (99mTc)TRODAT-1: a novel technetium-99m complex as a dopamine transporter imaging agent. Eur J Nucl Med 1997 ; 24 : 372.
54 Kurlan R, Kin MH, Gash DM. The time-course and magnitude of spontaneous recovery of parkinsonism by intracarotid administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydro pyridine to monkeys. Ann Neurol 1991 ; 29 : 677.
55 Lange KW. Clinical pharmacology of dopamine agonists in Parkinson's disease. Drugs Aging 1998 ; 13 : 381.
56 Langston JW, Forno LS, Rbert CS, Irwin I. Selective nigral toxicity after systemic administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in the squirrel monkey. Brain Res 1984 ; 292 : 390.
57 Leenders KL, Aquilonius SM, Bergstrom K, Bjurling P, Crossman AR, Eckernas SA, et al. Unilateral MPTP lesion in a rhesus monkey, effect on the striatal dopaminergic system assessed in vivo by PET using various novel tracers. Brain Res 1988 ; 445 : 61.
58 Leenders KL, Salmon E, Tyrrel P, Perani D, Brooks DJ, Sagar HJ, et al. The nigrostriatal dopaminergic system assessed in vivo by positron emission tomography in healthy volunteer subjects and patients with Parkinson's disease. Arch Neurol 1991 ; 47 : 1290.
59 Lindvall O, Sawle G, Widner H, Rothwell JC, Bjorklund A, Brooks D, et al. Evidence of long-term survival and function of dopaminergic grafts in progressive Parkinson's disease. Ann Neurol 1994 ; 32 : 172.
60 Logan J, Fowler JS, Volkow ND, Wolf AP, Dewey SL, Schlyer DJ, et al. Graphical analysis of reversible radioligand binding from time-activity measurements applied to (N-11C-methyl)-(-)-cocaine PET studies in human subjects. J Cereb Blood Flow Metab 1990 ; 10 : 740.
61 Logan J, Wolf AP, Shiue CY, Fowler, JS. Kinetic modeling of receptor-ligand binding applied to positron emission tomographic studies with neuroleptic tracers. J Neurochem 1987 ; 48 : 73.
62 Luxen A, Perlmutter M, Bida GT, van Moffaert G, Cook JS, Satyamurthy N, et al. Remote, semiautomated production of 6-(18F)fluoro-L-dopa for human studies with PET. Appl Radiat Isotopes 1990 ; 41 : 275.
63 Madras BK, Fahey MA, Bergman J, Canfield DR, Spealman RD. Effects of cocaine and related drugs in nonhuman primates; I.[3H] Cocaine binding sites in caudate-putamen. J Pharmacol Exp Ther 1989 ; 251 : 131.
64 Madras BK, Gracz LM, Fahey MA, Elmaleh D, Meltzer PC, Liang AY, et al. Altropane, a SPECT or PET imaging probe for dopamine neurons: III. Human dopamine transporter in postmortem normal and Parkinson's diseased brain. Synapse 1998 ; 29 : 116.
65 Maloteaux JM, Vanisberg MA, Laterre C, Javoy-Agid F, Agid Y, Ladurin PM. 3H-GBR 12935 binding to dopamine uptake sites, subcellular localization and reduction in Parkinson's disease and progressive supranuclear palsy. Eur J Pharmacol 1988 ; 156 : 331.
66 Marsden CD. Parkinson disease. Postgrad Med J 1992 ; 68 : 538.
67 Martin WR, Stoessl AJ, Adam MJ, Ammann W, Bergstrom M, Harrop R, et al. Positron emission tomography in Parkinson's disease: glucose and DOPA metabolism. Adv Neurol 1989 ; 45 : 95.

68 Meltzer PC, Liang AY, Brownell AL, Elmaleh DR, Madras BK. Substituted 3-phenyltropane analogs of cocaine: synthesis, inhibition of binding at cocaine recognition sites, and positron emission tomography imaging. J Med Chem 1993 ; 36 : 855.
69 Mintun MA, Raichle ME, Kilbourn MR, Wooten GF, Welch MJ. A quantitative model for in vivo assessment of drug binding sites with positron emission tomography. Ann Neurol 1984 ; 15 : 217.
70 Mizuno Y, Hattori N, Matsumine H. Neurochemical and neurodegenerative correlates of Parkinson's disease. J Neurochem 1998 ; 71 : 893.
71 Moeller JR, Eidelberg D. Divergent expression of regional metabolic topographies in Parkinson's disease and normal aging. Brain 1997 ; 120 : 2197.
72 Ogawa N. Early introduction of dopamine agonists in the long-term treatment of Parkinson's disease. Neurology 1998 ; 51 : S13.
73 Patlak CS, Blasberg RG. Graphical evaluation of blood-to-brain transfer constants from multiple-time uptake data. J Cereb Blood Flow Metab 1985 ; 5 : 584.
74 Piccini P, Turjanski N, Brooks DJ. PET studies of the striatal dopaminergic system in Parkinson's disease (PD). J Neural Transm Suppl 1995 ; 45 : 123.
75 Prochiantz A, di Porzio U, Kato A, Berger B, Glowinski J. In vitro maturationof mesencephalic dopaminergic neurons from mouse embryos is enhanced in presence of their striatal target cells. Proc Natl Acad Sci USA 1979 ; 76 : 5387.
76 Prochiantz A, Daguet MC, Herbet A, Glowinski J. Specific stimulation of in vivo maturation of mesencephalic dopaminergic neurons by striatal membranes. Nature 1981 ; 293 : 570.
77 Sadzot B, Price JC, Mayberg HS, Douglass KH, Dannals RF, Lever JR, et al. Quantification of human opiate receptor concentration using high and low specific activity [11C]diprenorphine and positron emission tomography. J Cereb Blood Flow Metab 1991 ; 11 : 204.
78 Schapira AHV. Neurotoxicity and the mechanisms of cell death in Parkinson's disease. In: Battistin L, ed. Advances in Neurology. Philadelphia: Lippincott-Raven; 1996. p. 161.
79 Tanner CM, Ottman R, Goldman SM, Ellenberg J, Chan P, Mayeux R, et al. Parkinson disease in twins : an etiologic study. J Amer Med Assoc 1999 ; 281 : 341.
80 Tolosa E, Marti MJ, Valldeoriola F, Molinuovo JL. History of levodopa and dopamine agonists in Parkinson's disease treatment. Neurology 1998 ; 50 : S2.
81 Wullner U, Pakzaban P, Brownell AL, Hantraye P, Burns L, Shoup T, et al. Dopamine terminal loss and onset of motor symptoms in MPTP-treated monkeys: a positron emission tomography study with ^{11}C-CFT. Exper Neurol 1994 ; 126 : 305.
82 Zawada WM, Zastrow DJ, Clarkson ED, Adams FS, Bell KP, Freed CR. Growth factors improve immediate survival of embryonic dopamine neurons after transplantation into rats. Brain Res 1998 ; 786 : 96.

In press

Dr. Ole Isacson
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Improved surgical cell therapy in Parkinson's disease: Physiological basis and new transplantation methodology

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1. Physiological basis for treatments of parkinsonism

In the classical neuropathological description of Parkinson's Disease (PD) there is abnormal degeneration and dysfunction of the dopaminergic (DA) neurons in the substantia nigra pars compacta region; with associated DA axonal and synaptic loss in the striatum, subthalamic nucleus and substantia nigra pars reticulatae. This in effect leaves the patient with signs of resting tremor, bradykinesia, rigidity and inability to initiate movements unless L-dopa treated. Other parallel pathologies may be present but the dopaminergie degeneration probably accounts for the motoric dysfunction.

The ability to observe both physiology and function in small areas within the brain is now possible with high resolution PET and MR imaging techniques(1-3). The potential use of PET as a research tool in movement disorders has been demonstrated in studies of brain dopamine function (4) and glucose metabolism associated with movement disorders(5-7). Recently, high resolution PET imaging has been widely used in studies with animal models of Parkinson's disease(8-15). In addition, advances in receptor studies (8, 10, 16, 17) and magnetic resonance spectroscopy of neurodegeneration (3, 15, 18-20) provide specific functional neurochemical information which may be of value in determining prognosis and therapy. In PD, the finding of changes in metabolic activity as determined by positron emission tomography (PET), in pre-SMA and PM cortices; are intriguing(21). What actually may explain the signs of PD at the physiological circuitry level, is the synchronization of neuronal pallidal (GPE and GPI) output signals as a result of the loss of DA tonic input to the putamen together with

a *reduced* thalamic input to the SMA and PM cortices (22) (see Figure 1-2). An apparent recruitment of more cortical regions than normal and the increased and widespread activation of PM and SMA associated cortices suggest that these structures are compensating for the abnormal input; to be able to activate the motor cortex for initiation of the movement (see Figure 1). Evidence for this view comes from pallidotomy studies(22) in which the loss of PD signs (tremor, rigidity and L-dopa induced dyskinesias) is reflected in a more localized metabolic activity, as well as appropriate activation in motor association cortex ((21) and Yoland Smith, personal communication). Based on all of these data and relevant circuitry of motor system, we hypothesize that full DA reinnervation, by cell transplantation or regeneration, will rebalance this motor system.

Studies in PD patients and primate PD models have postulated that nigrostriatal DA deficiency leads to decreased inhibitory activity from putamen to the internal segment of globus pallidus(23-25). Resulting inhibitory outflow from globus pallidus suppresses the motor thalamus reducing activation of cerebral motor cortex system and creates the signs of PD. After transplantation therapy, putaminal inhibitory action of globus pallidus is expected to recover. Longitudinal studies after different transplantation regimen with dopamine fetal cells is necessary to determine the underlying biological mechanism of the therapeutic effects on movement initiation.

Insert Figure 1 here:

Figure 1. (a) A schematic circuit of network diagram highlighting motor systems interactions. The results of STN lesions (and pallidotomy) may simply provide a release of movement control via the indirect pathway (CP - GPi and GPE via STN regulation of GPi inhibition of thalamic VA/VL). In this way the clinical signs of abnormally reduced or enhanced dopamine release in the CP can be eliminated, for example, the L-dopa induced dyskinesias typically seen in advanced PD patients virtually disappear after pallidotomy or STN modulation. Reports indicate that such advanced PD patients can sustain excessive DA activation of CP and remaining A10 DA neuronal systems (for example, nucleus accumbens) after STN stimulation or pallidotomy. These observations suggest that the indirect basal ganglia loop either blocks cortical motor-output (as in dopamine deficiency in PD) or create abnormal "oscillation" of cortical output (as in DA/L-dopa induced dyskinesias or Huntington's Disease).

Insert Figure 2 here:

Figure 2. A schematic network of the functional interactions of the motor systems in parkinsonism. The thick black lines show enhanced activation. The thick dark gray lines are enhanced inhibition. The broken lines are reduced inhibitory (GPE) or excitatory (Th) activity.

In the medical history of Parkinson's disease, James Parkinson's description of the disease in 1817 did not relieve the patients of their suffering until the early 1950s, when observations were made about unilateral improvements of PD signs contralateral to the subcortical stroke. Stereotactic thalamotomies and, subsequently, pallidotomies were performed as a rational intervention simulating this therapeutic effect. Surgical trials were fairly extensive and, in many cases, provided long term improvements and reductions in the patients' signs over several years (e.g. see volumes in Acta Psychiatrica Neurologica Scandinavica, 1960). The thermal lesions of such type were, however, superceded by the discovery that the dopamine system was *prima culpa* in Parkinson's disease. Through findings by Arvid Carlson's team in Sweden, Oleh Hornykiewics in Austria; the systemic pharmacological dosing and delivery of L-dopa was initiated to patients (e.g., Tolosa et al. 1998(26)). This became the mainstay treatment, and only after some time did it become evident that this precursor treatment (overcoming the dopamine rate-limiting enzyme tyrosine hydroxylase) in dopamine neurons would not be a permanent relief for the patients(26-29).

A novel rational idea then emerged from scientists working in neurobiology and cell culture: to replace lost dopamine neurons through neural transplantation(30, 31). Since animal experiments in the early 1980s and through the beginning of exploratory clinical trials in the late 1980s; the development of a new therapy involving fetal transplantation has reached some spectacular results(32-37) as well as some evidence of the need for further refinement(38, 39). In PD, neural transplantation potentially will

replace the missing dopamine neurons and provide an endogenous source (rather than a drug source) of dopamine in the striatum and other dopamine-depleted regions. The last few years have provided clear evidence that dopamine cell transplant therapy additionally improves drug response to L-dopa(36, 37, 40). The L-dopa can be taken up by transplanted dopamine cells and be appropriately converted by the dopa-decarboxylase into dopamine and released in a physiological way into the anatomical target zones.

2. Combined use of DA pharmacological and cell therapies: Can the neuronal replacement enhance L-dopa responses and reduce L-dopa induced dyskinsesias in Parkinson's disease?

After neural transplantation of fetal dopamine cells to PD patients, (when appropriate methods are used for transplantation) the vast majority of patients show a reduction in L-dopa-induced abnormal dyskinesias and dystonias in the "on" phase, as well as percent time spent in "off"(35-37). These findings are correlated with the presence of surviving dopaminergic grafts. Between 4 and 12 months after transplantation, up to 10 patients worldwide have reduced their L-dopa usage to zero, in association with 50-80% reductions in prior symptoms as assessed by the clinical rating scale (e.g. UPDRS). Cell replacement therapy is, however, in its infancy. This treatment modality is indeed novel; and therefore requires experimentation to become useful and understandable in a neurobiological context(39). The symptomatic recovery in some individual patients from parkinsonism with grafting of fetal neural cells is truly

remarkable. However, the six or seven research teams using this methodology around the world are in a sense artisans; in that they are using different techniques and clearly have different success rates(35). Moreover, most of these research teams are improving their technology and transplantation techniques every year. Thus, the skepticism levied at some of their results and inconsistencies is understandable. We have reviewed many active programs in North America and Europe, and find that the most reliable finding after transplantation is some reduction of the use of L-dopa and a dramatic loss of dyskinesias and "on/off" phenomena with full L-dopa dose. The most likely explanation for this finding is that the biosynthetic machinery provided by the cells implanted will allow L-dopa, through dopamine decarboxylase and other synaptic vesicular transport mechanisms; to be released and regulated for constancy of concentration at the synaptic sites in the striatum or elsewhere. In this view, a compelling reason for neural transplantation is to provide needed relief for patients from drug side effects, which is a primary source for current pain and dysfunction. This interpretation of neural transplantation effects in PD also provides an understanding of the progressive improvements seen. The patients worldwide that have no need for L-dopa after transplantation all demonstrated a progressive improvement over a six-month to six-year period that is sustained(33, 35). Moreover, the degeneration seen of the *host* dopaminergic systems also continues with no apparent effect on the implanted cells. This becomes apparent when analyzing data from unilaterally transplanted patients (Lindvall et al.'s cohort in Lund, Sweden). In their initial patient-series, as the grafted side became functional, the contralateral side continued to degenerate(35). In this way, a form of hemiparkinsonism developed and one patient was relieved by subsequent bilateral

transplantation (35). This type of clinical case-by-case evidence is complemented by a solid base of research in rodents and monkeys that demonstrates that the CNS dopaminergic system can be repaired(41). Nonetheless, in clinical application the variability in procedures by different transplant teams (on nearly all parameters used in cell preparation, cell number and sites transplanted) as well as the lack of understanding of the large number of individual forms of parkinsonism among PD patients, make comparisons and conclusions difficult.

In summary, starting with animal experiments in the early 1980s and through the beginning of exploratory clinical trials in the late 1980s the development of a new therapy involving fetal transplantation has reached some spectacular results(32-37) as well as much evidence for the need of further refinement(38, 39). Essentially, the treatment for PD with neural transplantation potentially will replace all the missing dopamine neurons and thereby provide an endogenous source (rather than a drug source) of dopamine in the striatum and other dopamine-depleted regions. The last few years have provided substantial evidence that dopamine cell transplant therapy additionally improves drug response(36, 37, 40). The L-dopa can be taken up by transplanted dopamine cells and be appropriately converted by the dopa-decarboxylase into dopamine and released in a physiological way into the anatomical target zones. As will be outlined in the next section, the most recent research for transplantation in PD has shown that more targets than striatum need to be transplanted for optimal anatomical and functional effects(42-48). We consider that triple stereotactic targeting including the striatum, the subthalamic nucleus and substantia nigra pars reticulata may be necessary for optimal

recovery in patients. This would achieve reinnervation of frontal (CPU and STN) and caudal output systems (the substantia nigra pars reticulata and brain stem/spinal systems. Preliminary studies suggest a rapid and complete restoration of function after such procedures(42-48).

3. Transplantation of dopamine cells: what are the appropriate cells and transplantation sites ?

Experimental work has established protocols for reliable dopamine cell transplant survival, outgrowth and function, as a rational idea for PD intervention derived from neurobiology and cell culture(30, 31). Many different strategies to improve the efficacy and survival of fetal ventral mesencephalic grafts have been described including treatment with growth factors(49, 50), antioxidants(51), and variations in cell dissociation techniques(52). The implantation procedure itself can also affect the outcome. Nikkah et al. have used a micrografting paradigm in rodents and shown that several implants of small volumes of cell suspension in the denervated striatum result in better functional recovery compared with animals receiving traditional macrograft implants(53).

Another important question in neural transplantation is the capacity for specific neuronal cell types to reinnervate selectively denervated host target regions(54-57). Transplanted embryonic neurons placed in denervated host targets display a relative specificity of fiber outgrowth into areas typical of their adult phenotype(54-57). The

dopaminergic neurons of the midbrain can be divided into various subsets distinguished by different immunoreactivity, for example, dopamine transporter, TH, calbindin and cholecystokinin (CCK)(54, 58). Some of these markers correspond to distinct projection patterns from the substantia nigra and have been used to provide evidence for the selective growth regulation of grafted tissue(54).

Fetal cell transplantation experiments generally, also makes discoveries possible for applications involving stem cells. Simply put, stems cells allow a more abundant generation of transplantable DA cells than sources from fetal primary cells. The current opportunities for PD therapy are limited by access to appropriate cells for transplantation. There are a number of choices beyond the use of human fetal cells. For example, xenogeneic fetal mesencephalic neurons, stem cells, immortalized cells or genetically modified non-neuronal or neuronal cells. Any of these choices are dependent on the state of research in each particular branch of cell biology. We have found that xenogeneic cells (pig) can be used both in animal models and patients with some success(57, 59). Nevertheless, we believe the aim of producing a reliable source of a very large number of dopamine cells will require a vigorous research programs for other cell types, such as stem cells. The use of pluripotent cells from the early blastula stage, which, if the differentiation steps were known and genetic modification possible, would provide a major source for transplantable dopamine cells. We have shown(60, 61) that differentiation of such stem cell grafts can lead to the production of nerve cells, including dopaminergic neuronal phenotypes. Nevertheless, the realistic use of a stem cell source can only be achieved, in our view, after considerable experimentation, which will

probably be preceded in the clinic by improvements in the standard fetal cell transplantation paradigm. This is important, because data from a number of research groups over the last decade demonstrate that fetal cell transplantation can help parkinsonian patients, while there is still considerable room for technical improvements. Many neurosurgeons and neurologists have expressed the view that the neural cell implant treatment could supercede any currently available methodology, such as physiological stimulation or lesioning procedures; pallidotomy or deep brain stimulation.

Surgical treatments in PD have seen a renaissance by electrophysiological intervention, using deep brain stimulation such as pallidal or subthalamic high frequency stimulation. By most accounts, these interventions provide relief from symptoms through depolarization, causing subsequent inhibition or activation of thalamic nuclei and their outflow to the cortex, or through subcortical output systems(22). This type of approach is based on an understanding of the circuitry involved in PD(see Fig.2). Also neural cell transplantation, can be applied to this specific circuitry, but as a form of "repair" in regions involved in the ablative electrical stimulations, since these regions are dopaminoceptive. Through dopamine synapse replacement by transplants, improvements to existing patient responses to L-dopa could be obtained. The DA released in the STN and SNr are of exceptional importance for normal motor function(44-48). The previous focus on transplantation to the dopaminoceptive caudate-putamen, while useful, may have lacked the necessary DA replacement needed for normal function of the STN output to mesencephalic locomotor regions and VA/VL thalamus. (Fig. 3)

Insert Figure 3 here:

Figure 3. Transplantation sites in putamen, substantia nigra and subthalamic nucleus: Two sites in the putamen will be transplanted (arrows) and depending on the paradigm, there will be tranplantation to the subthalamic nucleus and the substantia nigra (arrows) (see text).

Indeed, research towards transplantation in Parkinson's disease has recently indicated that additional targets to the striatum need to be transplanted for optimal anatomical and functional effects to occur(42-48). Thus, further research is warranted to determine the effects of multiple stereotactic targets including the striatum, the subthalamic nucleus and substantia nigra pars reticulata. This will allow DA reinnervation of the direct (nigro-striatal) and indirect pathway (including STN) of frontal and caudal output systems (to the substantia nigra pars reticulata and possibly beyond in the brain stem). The VTA (A10) DA input to layer 6 is relatively intact in PD. Preliminary data, including from our own laboratories, indicate that fetal (or equivalent) dopaminergic neurons can innervate all DA zones depleted in PD. Experiments in progress investigate the specific contribution of additional targets to functional recovery by addressing if multiple target grafting (CPU, SNr, STN) will restore function in a PD primate model.

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REFERENCES

1. Brownell GL, Burnham CA, Stearns CW, Chesler DA, Brownell A-L, Palmer M. Development in high-resolution positron emission tomography at MGH. Int J Img Systems Technol 1989;1:207-217.

2. Derenzo SE, Hueasman RH, Cahoon JL. A positron tomograph with 600 BGO crystals and 2.6 mm resolution. IEEE Trans Nucl Sci 1988;35:659-664.

3. Jenkins BG, Brouillet E, Chen YI et al. Non-invasive neurochemical analysis of focal excitotoxic lesions in models of neurodegenerative illness using spectroscopic imaging. J Cereb Blood Flow Metab 1996;16:450-461.

4. Piccini P, Turjanski N, Brooks DJ. PET studies of the striatal dopaminergic system in parkinson's disease (PD). J Neural Transm Suppl 1995;45:123-131.

5. Hirato M, Ohye C, Takahashi A, Negishi M, Shibasaki T. Study on the function of basal ganglia and frontal cortex using depth microrecording and PET scan in relation

to the outcome of pallidotomy for the treatment of rigid-akinesia-type Parkinson's disease. Stereotact Funct Neurosurg 1997;69:86-92.

6. Antonini A, Leenders KL, Vontobel P et al. Complementary PET studies of striatal neuronal function in the different diagnosis between multiple system atrophy and Parkinson's disease. Brain 1997;120:2187-2195.

7. Moeller JR, Eidelberg D. Divergent expression of regional metabolic topographies in Parkinson's disease and normal aging. Brain 1997;120:2197-2206.

8. Hantraye P, Brownell A-L, Elmaleh DR et al. Dopamine fiber detection by [11C]-CFT and PET in a primate model of parkinsonism. NeuroReport 1992;3:265-268.

9. Wullner U, Pakzaban P, Brownell A-L et al. Dopamine terminal loss and onset of motor symptoms in MPTP-treated monkeys: A positron emission tomography study with 11C-CFT. Exp Neurol 1994;126:305-309.

10. Brownell A-L, Elmaleh DR, Meltzer P et al. Cocaine congeners as PET imaging probes for dopamine terminals in normal and MPTP induced parkinsonism in nonhuman primate brain. J Nucl Med 1996;37:1186-1192.

11. Jordan S, Eberling JL, Bankiewicz KS et al. 6-[18F]fluoro-L-m-tyrosine: metabolism, positron emission tomography kinetics, and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine lesions in primates. Brain Res 1997;750:264-276.

12. Eberling JL, Jagust WJ, Taylor S et al. A novel MPTP primate model of Parkinson's disease: neurochemical and clinical changes. Brain Res 1998;805:259-262.

13. Doudet DJ, Chan GL, Holden JE et al. 6-[18F]Fluoro-L-DOPPA PET studies of the turnover of dopamine in MPTP-induced parkinsonism in monkeys. Synapse 1998;29:225-232.

14. Brownell A-L, Livni E, Galpern W, Isacson O. PET imaging of dopamine terminals in a rat transplant model of Parkinson disease. Ann Neurol 1998;43:387-390.

15. Brownell A-L, Jenkins BG, Elmaleh DR, Deacon TW, Spealman RD, Isacson O. Combined PET/MRS brain studies show dynamic and long-term physiological changes in a primate model of Parkinson disease. Nature Med 1998;4:1308-1312.

16. Frost JJ, Rosier AJ, Reich SG et al. Positron emission tomographic imaging of the dopamine transporter with 11C-WIN 35,428 reveals marked decline in mild Parkinson's disease. Ann Neurol 1993;34:423-431.

17. Ginovart N, Lundin A, Farde L et al. PET study of the pre- and post-synaptic dopaminergic markers for the neurodegenerative process in Huntington's disease. Brain 1997;120:503-514.

18. Holshouser BA, Komu M, Moller HE et al. Localized proton NMR spectroscopy in the striatum of patients with idiopathic Parkinson's disease: a Multicenter pilot study. Magn Reson Med 1995;33:589-594.

19. Guimareas AR, Schwartz P, Prakash MR et al. Quantitative nuclear magnetic resonance spectroscopic imaging of neuronal loss in excitotoxic rat brain model. Neuroscience 1995;69:1095-1101.

20. Federico F, Simone IL, Lucivero V et al. Proton magnetic resonance spectroscopy in Parkinson's disease and atypical parkinsonian disorders. Mov Disord 1997;12:903-909.

21. Grafton ST, Waters, C., Sutton, J., Lew, M.F., Couldwell, W. Pallidotomy increases activity of motor association cortex in Parkinson's disease: a positron emission tomographic study. Ann Neurol 1995;37:776-783.

22. Lozano AM, Lang AE, Hutchison WD, Dostrovsky JO. New developments in understanding the etiology of Parkinson's disease and in its treatment. Curr Opin Neurobiol 1998;8:783-790.

23. Alexander GE, Crutcher, M.D., DeLong, M.R. Basal ganglia thalamo-cortical circuits: parallel substrates for motor, oculomotor, "prefrontal" and "limbic" functions. Prog Brain Res 1990;85:119-146.

24. Filion M, Tremblay, L. Abnormal spontaneous activity of globus pallidus neurons in monkeys with MPTP-induced parkinsonism. Brain Res 1991;547:142-151.

25. Bergman H, Wichmann, T., Karmon, B., DeLong, M.R. The primate subthalamic nucleus. II. Neuronal activity in the MPTP model of parkinsonism. J Neurophysiol 1994;72:507-520.

26. Tolosa E, Marti MJ, Valldeoriola F, Molinuovo JL. History of levodopa and dopamine agonists in Parkinson's disease treatment. Neurology 1998;50:S2-10.

27. Marsden CD. Parkinson disease. Postgrad Med J 1992;68:538-543.

28. Lange KW. Clinical pharmacology of dopamine agonists in Parkinson's disease. Drugs Aging 1998;13:381-389.

29. Poewe W. Should treatment of Parkinson's disease be started with a dopamine agonist? Neurology 1998;51:S21-24.

30. Bjorklund A, Stenevi U. Reconstruction of the nigrostriatal dopamine pathway by intracerebral nigral transplants. Brain Res 1979;177:555-560.

31. Perlow M, Freed W, Hoffer B, Seiger A, Olson L, Wyatt R. Brain grafts reduce motor abnormalities produced by destruction of nigrostriatal dopamine system. Science 1979;204:643-647.

32. Hong M, Mendez I. Double grafting of human fetal dopamine cells restores function and antomy of the nitrostriatal pathway in the rodent model of Parkinson's disease. Exp Neurol Suppl 1999;in press.

33. Mendez I, Dagher A, Hong M. Transplantation of fetal dopamine cells hibernated in GDNF: From animal studies to clinical trials. Exp Neurol Suppl 1999;in press.

34. Lindvall O, Sawle G, Widner H et al. Evidence for long-term survival and function of dopaminergic grafts in progressive Parkinson's disease. Ann Neurol 1994;2:172-180.

35. Lindvall O. Update on fetal transplantation: the Swedish experience. Movement Dis 1998;13:83-87.

36. Freeman TB, Olanow CW, Hauser RA et al. Bilateral fetal nigral transplantation into the postcommissural putamen in Parkinson's disease. Ann Neurol 1995;38:379-388.

37. Wenning G, Odin P, Morrish P et al. Short- and long-term survival and function of unilateral intrastriatal dopaminergic grafts in Parkinson's disease. Ann Neurol 1997;42:95-107.

38. Freed CR, Breezr RE, Rosenberg NL et al. Survival of implanted fetal dopamine cells and neurologic improvement 12 and 46 months after transplantation for Párkinson's disease. New Engl J Med 1992;327:1549-1555.

39. Itakura T, Nakai E, Nakao N, Nakai K. Transplantation of neural tissue into the brain--a new therapeutic modality for the 21st century. Neurol Med Chir (Tokyo) 1998;38:756-762.

40. Kordower JH, Freeman TB, Snow BJ et al. Neuropathological evidence of graft survival and striatal reinnervation after the transplantation of fetal mesencephalic tissue in a patient with Parkinson's disease. New Engl J Med 1995;332:1118-1124.

41. Isacson O, Deacon TW. Neural transplantation studies reveal the brain's capacity for continuous reconstruction. Trends Neurosci 1997;20:477-482.

42. Mendez I, Sadi D, Hong M. Reconstruction of the nigrostriatal pathway by simultaneous intrastriatal and intranigral dopaminergic transplants. J Neurosci 1996;15:7216-7227.

43. Winkler C, Bentlage C, Nikkhah G, Samii M, Bjorklund A. Intranigral transplants of GABA-rich striatal tissue induce behavioral recovery in the rat Parkinson model and promote the effects obtained by intrastriatal dopaminergic transplants. Exp Neurol 1999;155:165-186.

44. van Horne CG, Mahalik T, Hoffer B et al. Behavioral and electrophysiological correlation of human mesencephalic dopaminergic xenograft function in the rat striatum. Brain Res Bulletin 1990;25:325-334.

45. Stromberg I, van Horne C, Bygdeman M, Weiner N, Gerhardt GA. Function of intraventricular human mesencephalic xenografts in immunosuppressed rats: electrophysiological and neurochemical analysis. Exp Neurol 1991;112:140-152.

46. van Horne CG, Hoffer BJ, Stromberg I, Gerhardt GA. Clearance and diffusion of locally applied dopamine in normal and 6-hydroxydopamine-lesioned rat striatum. J Pharmacol Exp Ther 1992;263:1285-1292.

47. van Horne CG, Eken S, Henry S, Hoffer BJ, Granholm AC. Nigral and striatal grafts into the subthalamic nucleus of 6-OHDA lesioned rats. The 6th Intl Neural Transplant Meeting Abstracts 1997;p. 2.

48. van Horne CG, Eken S, Henry S, Hoffer BJ, Granholm AC. Nigral and striatal grafts to the subthalamic nucleus in an animal model of Parkinson's disease. The World Society for Stereotactic and Functional Neurosurgery Abstracts 1997;98A-03:p. 75.

49. Haque NSK, Hlavin, M-L., Fawcett, J.W., Dunnett, S.B. The neurotrophin NT4/5, but not NT3, enhances the efficacy of nigral grafts in a rat model of Parkinson's disease. Brain Res 1996;712:45-52.

50. Mott JL, Eken, S., Bowenkamp, K., Gerhardt, G.A., Henry, S., Hoffer, B.J., Granholm, A-Ch. Effects of glial cell line-derived neurotrophic factor on dopaminergic transplants to the 6-OHDA denervated striatum. Soc Neurosci Abst 1996;22:1492.

51. Grasbon-Frodl EM, Nakao, N., Brundin, P. The Lazaroid U-83836E improves the survival of rat embryonic mesencephalic tissue stored at 4C and subsequently used for cultures or intracerebral transplantation. Brain Res Bulletin 1996;39:341-347.

52. Barker RA, Fricker, R.A., Abrous, D.N., Fawcett, J., Dunnett, S.B. A comparative study of preparation techniques for improving the viability of nigral grafts using vital stains, in vitro cultures and in vivo grafts. Cell Transplant 1995;4:173-200.

53. Nikkah G, Cunningham, M.G., Jodicke, A., Knappe, U., Bjorklund, A. Improved graft survival and striatal reinnervation by microtransplantation of fetal nigral cell suspensions in the rat Parkinson model. Brain Res 1994;633:133-143.

54. Schultzberg M, Dunnett, S.B., Bjorklund, A., Stenevi, U., Hokfelt, T., Dockray, G.J., Goldstein, M. Dopamine and cholecystokinin immunoreactive neurones in mesencephalic grafts reinnervating the neostriatum: evidence for selective growth regulation. Neuroscience 1984;12:17-32.

55. Nilsson OG, Clarke, D.J., Brundin, P., Bjorklund, A. Comparison of grówth and reinnervation properties of cholinergic neurons from different brain regions grafted to the hippocampus. J Comp Neurol 1988;268:204-222.

56. Isacson O, Deacon, T.W. Specific axon guidance factors persist in the mature rat brain: evidence from fetal neuronal xenografts. Neuroscience 1996;75:825-837.

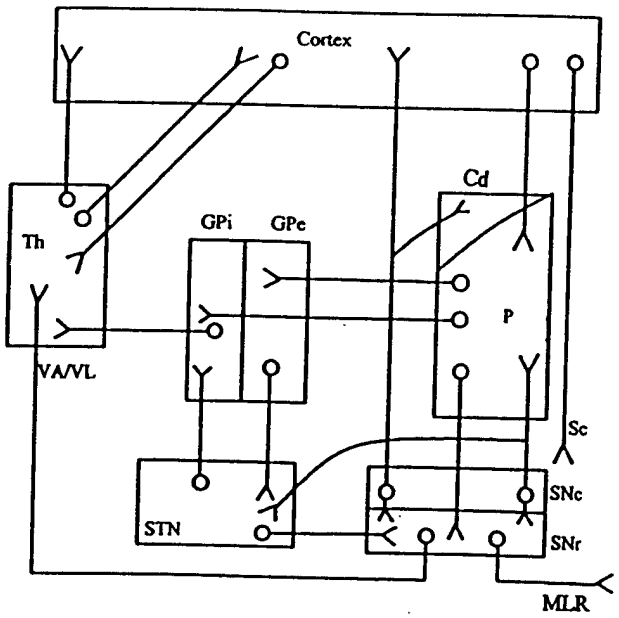
57. Isacson O, Deacon, T.W., Pakzaban, P., Galpern, W.G., Dinsmore, J., Burns, L.H. Transplanted xenogeneic neural cells for Parkinson's and Huntington's diseases exhibit remarkable axonal target specificity and distinct growth patterns of glial and axonal fibers. Nature Med 1995;1:1189-1194.

58. Haber SN, Ryoo, H., Cox, C., Lu, W. Subsets of midbrain dopaminergic neurons in monkeys are distinguished by different levels of mRNA for the dopamine transporter: comparison with the mRNA for the D2 receptor, tyrosine hydroxylase and calbindin immunoreactivity. J Comp Neurol 1995;362:400-410.

59. Deacon T, Schumacher J, Dinsmore J et al. Histological evidence of fetal pig neural cell survival after transplantation into a patient with Parkinson's disease. Nature Med 1997;130:350-353.

60. Deacon T, Dinsmore J, Costantini LC, Ratliff J, Isacson O. Blastula-derived stem cells can differentiate into dopaminergic and serotonergic neurons after transplantation. Exp Neurol 1998;149:28-41.

61. Isacson O. The neurobiology and neurogenetics of stem cells. Brain Pathol 1999;9:495-498.



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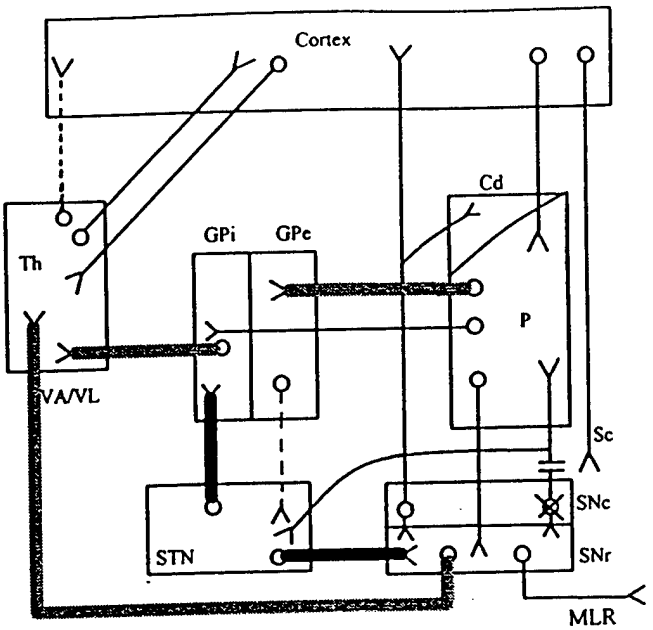

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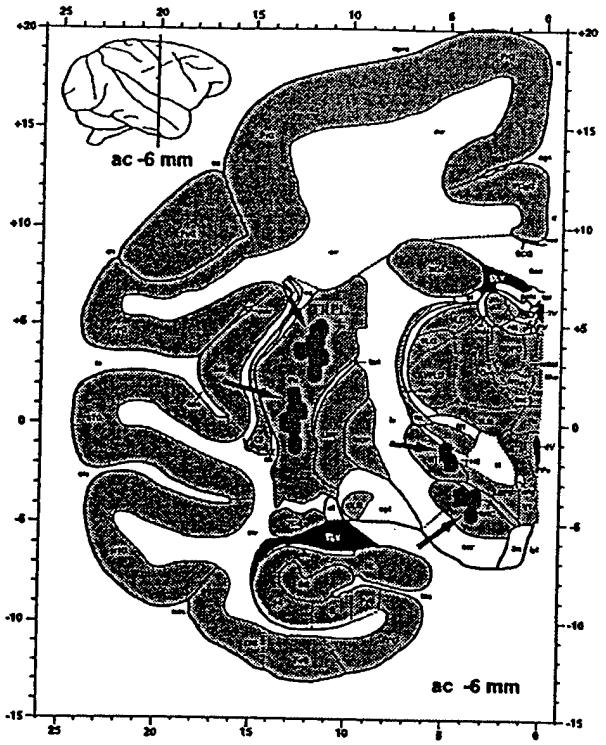

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CELL IMPLANTATION THERAPIES FOR PARKINSON'S DISEASE USING NEURAL STEM, TRANSGENIC OR XENOGENEIC DONOR CELLS

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ABSTRACT

A new therapeutic neurological and neurosurgical methodology involves cell implantation into the living brain in order to replace intrinsic neuronal systems, that do not spontaneously regenerate after injury, such as the dopaminergic (DA) system affected in Parkinson's disease (PD) and aging. Current clinical data indicate proof of principle for this cell implantation therapy for PD. Furthermore, the disease process does not appear to negatively affect the transplanted cells, although the patient's endogenous DA system degeneration continues. However, the optimal cells for replacement, such as highly specialized human fetal dopaminergic cells capable of repairing an entire degenerated nigro-striatal system, cannot be reliably obtained or generated in sufficient numbers for a standardized medically effective intervention. Xenogeneic and transgenic cell sources of analogous DA cells have shown great utility in animal models and some promise in early pilot studies in PD patients. The cell implantation treatment discipline, using cell fate committed fetal allo- or xenogeneic dopamine neurons and glia, is currently complemented by research on potential stem cell derived DA neurons. Understanding the cell biological principles and developing methodology necessary to generate functional DA progenitors is currently our focus for obtaining DA cells in sufficient quantities for the unmet cell transplantation need for patients with Parkinson's disease and related disorders.

The relatively new concept of replacing large numbers of degenerated neurons by implanting new cells into the adult brain has created a complementary therapeutic strategy to that of traditional pharmacological therapies for Parkinson's disease (PD). The specificity of cellular degeneration which occurs in PD (DA neurons of the SN), and the relatively major synaptic target region of these degenerating DA cells (the caudate, putamen and SN), have made PD the most accessible therapeutic application for neural cell implantation methodology.

Early clinical transplantation studies involved autologous transplantation of catecholamine-containing adrenal medulla cells [1, 2]. The absence of objective reductions of PD signs, the low adrenal medulla graft survival and the reported morbidity of patients reinforced the scientific rationale for using fetal neural donor cells instead. Cell implantation for PD using fetal DA cells is likely to improve greatly by scientific and technical advances. The development of brain cell transplantation with embryonic neurons and glia is innovative both from a technical and biological standpoint and will require much work to optimize. The scaling up of this method from rodents to primates has proved very challenging; particularly in obtaining an acceptable, abundant and reliable cell. In the initial series of clinical pilot transplant experiments performed in Europe, the first two PD patients did not show a meaningful recovery. Parallel technical and cell dose enhancements produced dramatically better results in the next two patients receiving unilateral fetal VM suspensions. MPTP-exposed patients received VM DA cell suspensions bilaterally into the striatum and this caused motor improvement in association with increased fluordopa uptake [3]. Recent data from the studies of Lindvall and colleagues indicate DA cell survival in patients for almost a decade after surgery, with meaningful clinical improvement [4]. The transplantation of non-dissociated human VM tissue pieces has also provided benefits to many patients [5, 6]. In this series of transplantation studies carried out by Olanow and colleagues in the US, autopsy from two bilaterally transplanted (6.5-9 week human fetal VM) patients who died 18-19 months after surgery showed over 200,000 surviving DA neurons, which reinnervated about 50% of the right putamen

and 25% of the left putamen [7]. Electron microscopy revealed axo-dendritic and occasional axo-axonic synapses between graft and host, and analysis of TH mRNA revealed higher expression within the fetal neurons than within the residual host nigral cells [7]. Autopsy of another patient in this surgical group showed over 130,000 surviving DA neurons, reinnervating almost 80% of the putamen [8]. Notably, both patients had shown major improvements in motor function and increases in fluorodopa uptake in the putamen on PET scanning.

An alternative source of fetal donor cells for clinical cell implantation therapy for neurodgenerative disease is xenogeneic. The remarkable homogeneity in cellular (neurons and glia) basic structure and function suggested that even discordant mammalian species (rodent into non-human primates) could effectively replace local synaptic function after cell loss in the adult brain [9, 10]. Such across-species cell transfer (xenotransplantation) allows a more standardized acquisition of larger quantities of appropriate fetal tissue than from human abortions. The immunological reaction of complement activated rejection and T-cell mediated responses leading to rejection of xenografts can in many ways be inhibited by immune suppression [11]. Transplantation studies in animals have shown survival, function, and afferent/efferent connections of xenogeneic cells when transplanted into animal hosts [12, 13] (and see reviews [14, 15]). In the first pilot-clinical trial, the transplantation of E27 porcine VM into the caudate and putamen on one side of the brain of twelve immunosuppressed PD patients produced some clinical improvements [16]. The overall results indicated that the scaling up problems, also seen with human fetal cells, were significant, further compounded by more vigorous immunological responses in primate and human hosts compared to laboratory rodents. One patient from this study died seven months after surgery from a pulmonary embolism; histological analyses using species-specific markers showed porcine neuron projection axons and forming synapses in the host brain. All three identified transplant sites contained DA neurons (a total of 630 DA neurons), and non-DA neurons expressing pig-specific neurofilament [17]. Pig glial cell, including astrocytes also survived in the patient's brain. Microglial and T-cell markers showed low reactivity in and around the pig cell graft perimeter.

The scientific foundation of cell implantation therapy for Parkinson's disease

Basic research involving cell implantation has made it abundantly clear that biological cell replacements strategies can provide the basis for reconstruction and repair of damaged or dysfunctional neuronal connections of the damaged or disease afflicted adult brain [18]. Functional effects of intrastriatal grafts of fetal DA cells have been illustrated in a range of animal behavioral tests [19-21]. The behavioral effects observed are dependent on the survival of DA neurons within the striatum, since grafting of other tissue produces no behavioral effects [22, 23], and removal of transplanted tissue [24] or immune rejection of transplanted neurons [25] reverses transplant-induced behavioral recovery in animal studies. Embryonic day (E) 12-17 fetal rat tissue [26], pig E 27-29 [13] and 6.5-9 weeks old human fetal tissue [5] ventral mesencephalic (VM) donor tissue neuronal exhibit survival and functional effects when transplanted into the adult dopamine depleted striatum. The minimum number of surviving transplanted DA neurons required for behavioral effect in rodent animal models is approximately 100-200 [12]. Using current micro-dissection techniques and cell preparation, only 10% of the transplanted VM cells are phenotypically DA, and only 1-20% of these DA neurons survive implantation depending on trophic and immunological factors [7, 17, 27-32]. Therefore as many as 10-15 fetal VM per patient may be required for sufficient survival and adequate DA synapse replacement [33].

Factors that are important for maturation and connectivity of DA neurons during normal ontogeny likely also influence development and integration of grafted embryonic tissue when placed in an adult host brain. Current methodology for fetal cell implantation in animal models and patients includes the transfer of numerous types of fetal neuronal and glial progentitor cells. Thus, the implanted neurons are transferred into the host brain with their own contemporaneous glial and angiogenic factor releasing cells, thereby providing a mileu that may contribute to the observed normal cell autonomous development of transplanted fetal VM cells. Adding appropriate trophic factors to fetal cell preparations can enhance survival and growth of implanted DA neurons into animal models of PD [28, 34-39]. The ability of fetal neurons to be placed into an ectopic region of an adult brain, survive, and extend neurites within this region is remarkable. The functional effects

of VM transplants into DA-depleted striatum is often correlated with degree of striatal reinnervation [26, 30]. However there is some limitation in the ability of the transplanted neurons to extend neurites in the adult brain. Even though the graft-induced elevations in tissue DA concentrations are substantial [40], values taken distant from the graft suggest that reinnervation of the whole striatum does not occur. The hypothesis for this sharp decline in density of DA fiber outgrowth is that age-dependent characteristics within the host brain alter outgrowth, since extensive outgrowth can be achieved when transplanted into immature (neonatal) host brain. Expression levels and patterns of adhesion molecules expressed by mature host brain are thought to be the culprits of this reinnervation-inhibitory effect. Allogeneic cell implantation into immature host brain shows robust neuronal and glial migration away from the transplant site and a high degree of integration and target-directed neurite outgrowth [18]. In contrast, fetal neural cells transplanted into mature brains show neuronal reaggregation around the implant site and less extensive axonal outgrowth into host brain, suggesting an age-dependent increase in inhibitory or decrease in growth-promoting processes. Clearly, both promoting and repulsive host factor and substrate activities influence axonal guidance and extension of transplanted developing neurons [18].

Data arguing against any absolute outgrowth-inhibitory properties of adult brain come from studies showing long-distance and target-specific axonal growth from human embryonic transplants into adult rat brain [41], as well as from porcine embryonic transplants into adult rat brain [13]. The species-specific markers used in our studies of fetal porcine transplants into adult immunosuppressed rat brain allowed comparison of donor glial fiber and donor axonal growth in different host brain regions, demonstrating their distinct trophic characteristics. Target zones in adult host gray matter were selectively innervated by embryonic donor axons normally destined to form synapses there, whereas donor glial fibers grew irrespective of any target orientation within white matter tracts [13]. Xenogenic pig axons branched profusely in gray matter target region and only rarely penetrated or crossed white matter tracts. DA fibers from transplants placed into the SN were found coursing up toward the striatum through myelinated fiber bundles, then branching into host gray matter. Notably, we found that the non-DA VM cells also grew toward distant gray matter

target zones, such as mediodorsal and ventral anterior thalamus. These data suggest that directional cues for axons, whether diffusible or substrate-bound, are provided by adult host target regions. Since porcine neural development continues four to five times longer than mouse, these axons may grow and make synapses for a longer time (with slower maturation) than that seen in rat-to-rat studies. These general differences are borne out in the time-course comparisons of functional recovery in rodent porcine-transplant recipients (8 weeks post-transplantation) as compared with allografts (4 weeks post-transplantation) [18].

Anatomical and cell type specification of dopamine neurons.

The current understanding of the maturation and phenotypic specializations of DA neurons located in the adult substantia nigra parallels the observations made of the development of committed fetal dopamine neurons placed as grafts into the adult CNS (Fig. 1). The molecular signaling necessary for the final morphological specializations and connectivity of the nigro-striatal DA system must therefore be largely intrinsic to the developing DA neurons; or alternatively, present in significant detail in the adult brain for this process to be completed in a normal way. On the post-synaptic host side; different regions of the striatum are associated with specific behaviors in rat: the dorsal striatum receives primary afferents from the motor areas of neocortex, and has been shown to be preferentially involved in rotational recovery after DA neuron transplantation [42]. In the intact rat, the subpopulation of nigral DA neurons from A9 SN which co-express AHD project their axons to the dorsal-lateral and rostral regions of the striatum. When transplanted into adult DA-denervated rat striatum, these AHD/TH neurons innervated this region of the DA-depleted striatum [18, 43]), showing a preferential reinnervation of the dorsolateral striatum corresponding to the normal projection pattern of AHD/TH neurons. Specific innervation by subsets of transplanted DA neurons was also demonstrated by Schultzberg, showing reinnervation of the DA-depleted striatum by the population of grafted A9 VM neurons lacking cholecystokinin (CCK) [44]. The CCK+ fibers were found in a narrow zone immediately adjoining the graft. These data suggest the presence of mechanisms which selectively favor the ingrowth of fibers from the appropriate DA

neuronal subset. Thus enrichment of the DA-neuron subpopulation which specifically expresses AHD may allow more appropriate reinnervation of striatum after transplantation, and influence the degree of functional recovery in PD [18] (Fig. 1).

Repair of synaptic function and regulated dopamine release after implantation of new dopamine neurons.

The most important factor in obtaining complete and sustained functional effects may be the presence of new synapses for biochemically and physiologically appropriate DA release in the host striatum. Embryonic DA neurons produce new connections with the mature host striatal neurons. Synaptic connections between transplanted VM cells and host cells, as well as afferents from host neurons to transplanted cells, have been extensively documented [45, 46]. Functional analyses indicate that pharmacological delivery into the striatum may not be as effective in ameliorating the motor symptom of PD, as regulated, synaptic release obtained with transplanted DA neurons [33]; When DA is directly administered into the ventricle of PD patients, serious psychosis can develop [47]. Even from a cell biological standpoint, the rationale for normal range DA release is illustrated by differential display experiments that show abnormal upregulation of over 10 genes within the striatum after abnormal DA exposure in vivo [48]. Complications associated with unregulated DA levels are obvious when observing effects of long-term L-dopa administration: as PD progresses, and the DA neuron degeneration continues, the unregulated formation of DA within the striatum and abnormal down-stream activity in the basal ganglia can lead to motor abnormalities such as dyskinesias. Physiologically appropriate DA functions can be achieved by DA neurons or, alternatively, cells which express the complete set of feedback elements required to regulate release and uptake of DA. Several studies have shown normalized metabolic activity throughout the basal ganglia after transplantation. Using cytochrome oxidase histochemistry as an indicator of neuronal metabolism in the 6-OHDA-lesioned rat; the lesion-induced increases in activity of the entopeduncular nucleus and SN reticulata were reversed by intrastriatal VM grafts, whereas the lesion-induced increases in globus pallidus and subthalamic nucleus were not affected by grafting

[49]. Similarly, in MPTP-treated monkey receiving VM transplants, DA cell implants increased the metabolic activity of the implanted striatum, particularly in the region of grafts containing greater numbers of DA neurons [50]. Positron emission tomography (PET) and carbon-11-labeled 2B-carbomethoxy-3B-(4-fluorophenyl)tropane (11C-CFT) have been utilized as markers for striatal presynaptic DA transporters in a unilateral lesion model in rat. In the lesioned striatum, the binding ratio was reduced to 15% to 35% of the intact side. After DA neuronal transplantation, behavioral recovery occurred only after the 11C-CFT binding ratio had increased to 75% to 85% of the intact side, revealing a threshold for functional recovery in the lesioned nigrostriatal system after neural transplantation[23]. Autoregulation of DA release and metabolism by intrastriatal grafts has been shown by in vivo microdialysis. Infusion of a non-selective DA agonist (apomorphine) reduced DA concentrations in the grafted striatum [12, 51], indicating auto-regulation of DA levels by transplanted cells. Evidence for the formation of functional synapses and appropriate DA regulation by transplanted fetal DA neurons comes from the observation that dyskinesias, expressed either as contraversive circling after repeated L-dopa injections in rodents [52] or L-dopa-induced dyskinesias in non-human primates are reduced after transplantation. These data indicate that DA levels within the transplanted striatum will be regulated in a functional manner by the transplanted DA neurons.

Potential use of stem cells for obtaining donor cell for transplantation.

Most living systems undergo continuous growth. There are many examples of cell division and differentiation for maintaining cell populations in adult human bodies; for example, the bone marrow that recruits stem cells capable of dividing into most of the cells necessary for blood and immune systems throughout life. Part of entire adult organs can be regenerated, such as the liver. Cells in the lining of the gut are shed on a daily basis with replacements growing in from layers below. In the skin, the basal cell layers of the dermis provides a plentiful source of growth; that also signifies a continuous growth process. These specialized cells can divide to maintain or increase growth of organ systems in the adult body. The recent fascination with the most

pluripotent of such cell; the so-called stem cells, illustrate a renewed interest and deepening molecular understanding of developmental biology. While for the last 60 years most text-books of embryology has detailed most biological sequences in the development of mammals, it is not until recently that a molecular and mechanistic data of cellular signaling pathways involved in cell fate and development of organ systems has been obtained. In addition, recent cloning experiments have illustrated that even mammalian adult cell nuclei (containing DNA) has the material for establishing all cells of a whole organism after transfer to a fertilized egg-cell. The fertilized oocyte goes through a few rounds of cell division and then the resulting cluster of cells (in the range of 250 cells; see Fig. 2) is capable of imbedding itself in the wall of the uterus in mammals. At this stage, each of the cells in the inner cell mass cluster is usually capable of forming any part, or the whole of the entire body plan. This type of cell is therefore denoted stem cell, or in this case, embryonic stem cells. From this initial group of stem cells, all other cells that form the living body are generated. The developmental sequential orchestration of the growth of the body into its specialized parts and unique form and function follows a strict pattern and sequence in the embryo and neonate. Nevertheless, as previously mentioned, in the adult organism, many cells with the body remain capable of division and growth into specialized cell systems. Recently, such divisible (yet non-malignant or carcinogenic) cells have gained increased attention. The idea that such multipotent cells present in the blood stream, or even in the brain, are still capable of multiple cellular fates has intrigued biologists and the public. In particular in the brain, in addition to the well-known fact that olfactory epithelium and a few other brain regions (including the dentate gyrus of the hippocampus) there may also be dividing cells capable of other types of growth or repair. Such continuous cell division may be necessary for maintenance and adaptive function of many cellular systems.

In experimentation, stem cell-like behavior has been observed from embryonic stem cells, growth factor-expanded neural progenitors, immortalized cell lines and embryonal carcinoma cells. Growth factor-expanded cells have been implanted into the adult brain, with survival of small cell clusters [53-55]. Immortalized cell lines have shown capacity to differentiate into several neuronal cell types when transplanted (for review, see [56]). The implantation of immortalized cells into

neonatal brain resulted in differentiation into neurons and glia with apparent region-specific morphology [57-60]. Notably, when transplanted into adult brain such immortalized cells (generated from embryonic striatum or hippocampus) are usually fated to form glia [61]. Brain implants of embryonic carcinoma cell lines have been shown to survive and grow as neurons when treated with retinoic acid [62-65]. We transplanted mouse D3 and E3 normal ES cells into adult mouse striatum and adult 6-OHDA-lesioned striatum, which spontaneously developed into neurons and other cells (Fig. 2). Many TH^+ neurons were found, while dopamine-ß-hydroxylase$^+$ cells were infrequent. Non-neuronal regions sometimes were immunoreactive for glial fibrillary acidic protein. Many neurons, including DA and 5-HT catecholaminergic cells, grew axons into the host brain. The axonal growth into gray matter was not abnormal, but did not resemble the five caliber fiber innervation seen in normal DA growth in the striatum [13, 66]. ES derived serotonergic neurons grew in a less restricted pattern than TH^+ neurons. Mouse D3 and E3 ES cells placed into mouse kidney capsule grew into similar neuronal phenotypes as those placed in the adult brain. These data suggest that neuralization is a possible default pathway, and occurs spontaneously if pre-gastrula cells are prevented from getting patterned signals from other embryonic cell layers [67]. This is not entirely surprising, given that the early gastrula ectodermal animal cap, normally destined to become epidermal tissue, will form neural tissue if disrupted [68]. There are known inducing factors discovered for epidermal differentiation during gastrulation, such as BMP4 [69]. Homozygous knock-out mice lacking functional BMP receptor (BMPR1) will not survive past gastrulation [70], a time when epidermis would normally form. Inhibitors of BMP4 or activin, such as noggin, follistatin, and chordin, from the Spemann organizer region, can cause ectopic formation of neural tissue Taken together, these findings indicate that disruption of these epidermis-inducing signals causes neural differentiation. Given that our experiment involved dissociated and expanded ES cells, this may be equivalent of such disruption. Nonetheless, it remains to be determined if other growth factors present in brain and kidney capsule can induce TH+ neurons. The absence of kidney formation in GDNF-knockout mice suggests that GDNF may play a role in both kidney and brain development [71]. While these ES cells form neurons of TH+ (putative DA) phenotypes

that extend axons, into the adult host striatum, such neurons have not yet been shown to create the kind of behavioral recovery seen with implantation of normal phenotypic fetal DA neurons (Isacson et al, unpublished observations).

In conclusion, there is a large unmet need for obtaining a donor cell source for clinical cell implantation to PD patients. While human fetal DA donor cells work in principle, as shown in human pilot studies, this cell source is not available or workable in a standard clinical environment. Analogous fetal DA donor cells from other animal species are potential alternatives to human fetal tissue. For example, the pig or rodent meso-striatal DA system also contains cell groups A8, A9, and A10 that differentiate into the homologous cell groups seen in humans and function after transplantation to the mature brain. Alternatively, functionally appropriate DA neurons could be derived from progenitor or stem cell populations. Moreover, genetic engineering and immortalization technology could be applied to progenitor and stem cells, in order to obtain sufficient numbers of DA neurons of appropriate design for cell transplantation to a large number of PD patients.

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REFERENCES

[1] Madrazo I, Leon, V., Torres, C.,. Transplantation of fetal substantia nigra and adrenal medulla to the caudate putamen in two patients with Parkinson's disease. New Engl J Med 1988;318:51.

[2] Backlund E, Granberg, P., Hamberger, B. Transplantation of adrenal medullary tissue to striatum in parkinsonism. J Neurosurg 1985;62:169-173.

[3] Widner H, Tetrud J, Rehncrona S, Snow B, Brundin P, Gustavii B. Bilateral fetal mesencephalic grafting in two patients with severe parkinsonism induced by MPTP. New Engl J Med 1992;327:1556-1563.

[4] Piccini P, Brooks DJ, Bjorklund A, Gunn RN, Grasby PM, Rimoldi O, Brundin P, Hagell P, Rehncrona S, Widner H, et al. Dopamine release from nigral transplants visualized in vivo in a Parkinson's patient. Nat Neurosci 1999;2:1137-1140.

[5] Freeman TB, Olanow CW, Hauser RA, Nauert M, Smith DA, Borlongan CV, Sanberg PR, Holt DA, Kordower JH, Vingerhoets FJG, et al. Bilateral fetal nigral transplantation into the postcommissural putamen in Parkinson's disease. Ann Neurol 1995;38:379-388.

[6] Freed CR, Breezr RE, Rosenberg NL, Schneck SA, Kriek E, Qi JX, Lone T, Zhang YB, Snyder JA, Wells TH, et al. Survival of implanted fetal dopamine cells and neurologic improvement 12 and 46 months after transplantation for Parkinson's disease. New Engl J Med 1992;327:1549-1555.

[7] Kordower J, Rosenstein J, Collier T, Burke M, Chen E, Li J, Martel L, Levey A, Mufson E, Freeman T, et al. Functional fetal nigral grafts in a patient with Parkinson's disease: chemoanatomic, ultrastructural, and metabolic studies. J Comp Neurol 1996;370:203-230.

[8] Kordower J, Freeman T, Chen E, Mufson E, Sanberg P, Hauser R, Snow B, Olanow C. Fetal nigral grafts survive and mediate clinical benefit in a patient with Parkinson's disease. Mov Disord 1998;13:383-393.

[9] Isacson O, Riche D, Hantraye P, Sofroniew MV, Maziere M. A primate model of Huntington's disease: cross-species implantation of striatal precursor cells to the excitotoxically lesioned baboon caudate-putamen. Exp Brain Res 1989;75:213-220.

[10] Hantraye P, Riche D, M. M, Isacson O. Intrastriatal grafting of cross-species fetal striatal cells reduces abnormal movements in a primate model of Huntington's disease. Proc Natl Acad Sci USA 1992;89:4187-4191.

[11] Pedersen E, Poulsen F, Zimmer J, Finsen B. Prevention of mouse-rat brain xenograft rejection by a combination therapy of cyclosporin A, prednisolone and azathioprine. Exp Brain Res 1995;106:181-186.

[12] Galpern WR, Burns LH, Deacon TW, Dinsmore J, Isacson O. Xenotransplantation of porcine fetal ventral mesencephalon in a rat model of Parkinson's disease: functional recovery and graft morphology. Exp Neurol 1996;140:1-13.

[13] Isacson O, Deacon TW, Pakzaban P, Galpern WR, Dinsmore J, Burns LH. Transplanted xenogeneic neural cells in neurodegenerative disease models exhibit remarkable axonal target specificity and distinct growth patterns of glial and axonal fibres. Nature Med 1995;1:1189-1194.

[14] Pakzaban P, Isacson O. Neural xenotransplantation: reconstruction of neuronal circuitry across species barriers. Neuroscience 1994;62:989-1001.

[15] Isacson O, Breakefield, X.O. Benefits and risks of hosting animal cells in the human brain. Nature Med 1997;3:964-969.

[16] Schumacher JM, Ellias SA, Palmer EP, Kott HS, Dinsmore J, Dempsey PK, Fischman AJ, Thomas C, Feldman RG, Kassissieh S, et al. Transplantation of embyonic porcine mesencephalic tissue in patients with PD. Neurology 2000;14:1042-1050.

[17] Deacon T, Schumacher J, Dinsmore J, Thomas C, Palmer P, Kott S, Edge A, Penney D, Kassissieh S, Dempsey P, et al. Histological evidence of fetal pig neural cell survival after transplantation into a patient with Parkinson's disease. Nature Med 1997;130:350-353.

[18] Isacson O, Deacon TW. Neural transplantation studies reveal the brain's capacity for continuous reconstruction. Trends Neurosci 1997;20:477-482.

[19] Brundin P, Duan, W., Saur, H. Functional effects of mesencephaic dopamine neurons and adrenal chromaffin cells grafted to the rodent striatum.New York: Raven Press, 1994:9-46. (Dunnett S, Bjorklund, A., ed. Functional Neural Transplantation; vol 2).

[20] Annett L. Functional studies of neural grafts in parkinsonian primates.New York: Raven Press, 1994:71-102. (Dunnett S, Bjorklund, A., ed. Functional Neural Transplantation; vol 2).

[21] Olsson M, Nikkhah G, Bentlage C, Bjorklund A. Forelimb akinesia in the rat Parkinson model: differential effects of dopamine agonists and nigral transplants as assessed by a new stepping test. J Neurosci 1995;15:3863-3875.

[22] Dunnett S, Hernandez T, Summerfield, Jones G, Arbuttnott G. Graft-derived recovery from 6-OHDA lesions: specificity of ventral mesencephalic graft tissue. Expl Brain Res 1988;71:411-424.

[23] Brownell AL, Livni E, Galpern W, Isacson O. In vivo PET imaging in rat of dopamine terminals reveals functional neural transplants. Annals of Neurology 1998;43:387-390.

[24] Bjorklund A, Dunnett S, Stenevi U, Lewis M, Iversen S. Reinnervation of the denervated striatum by substantia nigra transplants: functional consequences as revealed by pharmacological and sensorimotor testing. Brain Res 1980;199:307-333.

[25] Carder R, Snyder-Keller A, Lund R. Behavioral and anatomical correlates of immunologically induced rejection of nigral xenografts. J Comp Neurol 1988;277:391-402.

[26] Bjorklund A, Lindvall O, Isacson O, Brundin P, Wictorin K, Strecker R, Clarke D, Dunnett S. Mechanisms of action of intracerebral neural implants: Studies on nigral and striatal grafts to the lesioned striatum. Trends Neurosci 1987;10:509-516.

[27] Olanow CW, Kordower JH, Freeman TB. Fetal nigral transplantation as a therapy for Parkinson's disease. Trends Neurosci 1996;19:102-109.

[28] Yurek D, Lu W, Hipkens S, Wiegand S. BDNF enhances the functional reinnervation of the striatum by grafted fetal dopamine neurons. Exp Neurol 1996;137:105-118.

[29] Nakao N, Frodl E, Duan WM, Widner H, Brundin P. Lazaroids improve the survival of grafted embryonic dopamine neurons. Proc Natl Acad Sci 1994;91:12408-12412.

[30] Costantini LC, Snyder-Keller A. Co-transplantation of fetal lateral ganglionic eminence and ventral mesencephalon can augment function and development of intrastriatal transplants. Exp Neurol 1997;145:214-227.

[31] Brundin P, Isacson O, Gage F, Bjorklund A. Intrastriatal grafting of dopamine-containing neuronal cell suspensions: effects of mixing with target or non-target cells. Brain Res 1986;389:77-84.

[32] Nikkah G, Olsson M, Eberhard J, Bentlage C, Cunningham MG, Bjorklund A. A microtransplantation approach for cell suspension grafting in the rat Parkinson model: a detailed account of the methodology. Neuroscience 1994;53:57-72.

[33] Bjorklund A. Better cells for brain repair. Nature 1993;362:414-415.

[34] Zhou J, Bradford H, Stern G. Influence of BDNF on the expression of the dopaminergic phenotype of tissue used for brain transplants. Dev Brain Res 1997;100:43-51.

[35] Mayer E, Dunnett S, Fawcett J. Mitogenic effect of basic fibroblast growth factor on embryonic ventral mesencephalic dopaminergic neurone precursors. Dev Brain Res 1993;72:253-258.

[36] Johansson M, Friedemann M, Hoffer B, Stromberg I. Effects of glial cell line-derived neurotrophic factor on developing and mature ventral mesencephalic grafts in oculo. Exp Neurol 1995;134:25-34.

[37] Wang Y, Tien L, Lapchak P, Hoffer B. GDNF triggers fiber outgrowth of fetal ventral mesencephalic grafts from nigra to striatum in 6-OHDA-lesioned rats. Cell Tissue Res 1996;286:225-233.

[38] Rosenblad C, Martinez-Serrano A, Bjorklund A. Glial cell line-derived neurotrophic factor increases survival, growth and function of intrastriatal fetal nigral dopaminergic grafts. Neuroscience 1996;75:979-985.

[39] Zawada W, Zastrow D, Clarkson E, Adams F, Bell K, CR F. Growth factors improve immediate survival of embryonic dopamine neurons after transplantation into rats. Brain Res 1998;786:96-103.

[40] Rioux L, Gaudin D, Bui L, Gregoire L, DiPaolo T, Bedard P. Correlation of functional recovery after 6-OHDA lesion with survival of grafted fetal neurons and release of dopamine in the striatum of the rat. Neuroscience 1991;40:123-131.

[41] Wictorin K, Brundin P, Sauer H, Lindvall O, A B. Long distance directed axonal growth from human dopaminergic mesencephalic neuroblasts implanted along the nigrostriatal pathway in 6-hydroxydopamine lesioned adult rats. J Comp Neurol 1992;323:475-494.

[42] Dunnett S, Bjorklund A, Schmidt R, Stenevi U, Iverson S. Intracerebral grafting of neuronal cell suspensions. IV. Behavioral recovery in rats with unilateral 6-OHDA lesions in different brain sites. Acta Physiol Scand 1983;522:29-37.

[43] Haque N, LeBlanc C, Isacson O. Differential dissection of the rat E16 ventral mesencephalon and survival and reinnervation of the 6-OHDA-lesioned striatum by a subset of aldehyde dehydrogenase-positive TH neurons. Cell Transplant 1997;6:239-248.

[44] Schultzberg M, Dunnett S, Bjorklund A, Stenevi U, Hokfelt T, Dockray G, Goldstein M. Dopamine and cholecystokinin immunoreactive neurons in mesencephalic grafts reinnervating the neostriatum: evidence for selective growth regulation. Neuroscience 1984;12:17-32.

[45] Mahalik T, Finger T, Stromberg I, Olson L. Substantia nigra transplants into denervated striatum of the rat: ultrastructure of graft and host interconnections. J Comp Neurol 1985;240:60-70.

[46] Doucet G, Murata Y, Brundin P, Bosler O, Mons N, Geffard M, Ouimet C, Bjorklund A. Host afferents into intrastriatal transplants of fetal ventral mesencephalon. Exp Neurol 1989;106:1-19.

[47] Venna N, Sabin T, Ordia J, Mark V. Treatment of severe Parkinson's disease by intraventricular injection of dopamine. Applied Neurophysiol 1984;47:62-64.

[48] Gerfen C, Keefe K, Steiner H. Dopamine-mediated gene regulation in the striatum. Adv Pharmacol 1998;42:670-673.

[49] Nakao N, Ogura M, Nakai K, Itakura T. Intrastriatal mesencephalic grafts affect neuronal activity in basal ganglia nuclei and their target structures in a rat model of Parkinson's disease. J Neurosci 1998;18:1806-1817.

[50] Collier T, Redmond DJ, Roth R, Elsworth J, Taylor J, Sladek JJ. Metabolic energy capacity of dopaminergic grafts and the implanted striatum in parkinsonian nonhuman primates as visualized with cytochrome oxidase histochemistry. Cell Transplant 1997;6:135-140.

[51] Strecker R, Sharp T, Brundin P, Zetterstrom T, Ungerstedt U, Bjorklund A. Autoregulation of dopamine release and metabolism by intrastriatal nigral grafts as revealed by intracerebral dialysis. Neuroscience 1987;22:169-178.

[52] Gaudin D, Rioux L, Bedard P. Fetal dopamine neuron transplants prevent behavioral supersensitivity induced by repeated administration of L-Dopa in the rat. Brain Res 1990;506:166-168.

[53] Svendsen C, Caldwell M, Shen J, Borg M, Rosser A, Tyers P, Karmio lS, Dunnett S. Long-term survival of human central nervous system progenitor cells transplanted into a rat model of Parkinson's disease. Exp Neurol 1997;148:135-146.

[54] Svendsen C, Clarke D, Rosser A, Dunnett S. Survival and differentiation of rat and human epidermal growth factor-responsive precursor cells following grafting into the lesioned adult central nervous system. Exp Neurol 1996;137:376-388.

[55] Minger S, Fisher L, Ray J, Gage F. Long-term survival of transplanted basal forebrain cells following in vitro propagation with fibroblast growth factor-2. Exp Neurol 1996;141:12-24.

[56] Martinez-Serrano A, Bjorklund, A. Immortaized neural progenitor cells for CNS gene transfer and repair. Trends Neurosci 1997;20:530-538.

[57] Renfranz PJ, Cunningham MG, McKay RDG. Region-specific differentiation of the hippocampal stem cell line HiB5 upon implantation into the developing mammalian brain. Cell 1991;66:713-729.

[58] Ryder EF, Snyder EY, Cepko CL. Establishment and characterization of multipotent neural cell lines using retrovirus vector-mediated oncogene transfer. J Neurobiol 1990;21:356-375.

[59] Snyder EY, Deitcher DL, Walsh C, Arnold-Aldea S, Hartwieg EA, Hartwieg LCC. Multipotent neural cell lines can engraft and participate in development of mouse cerebellum. Cell 1992;68:33-51.

[60] Cattaneo E, Magrassi L, Butti G, Santi L, Giavazzi A, Pezzotta S. A short term analysis of the behavior of conditionally immortalized neuronal progenitors and primary neuroepithelial cells implanted in the fetal rat brain. Dev Brain Res 1994;83:197-208.

[61] Lundberg C, Martinez-Serrano A, Cattaneo E, McKay RDG, Björklund A. Survival, integration and differentiation of neural stem cell lines after transplantation to the adult rat striatum. Exp Neurol 1997;145:342-360.

[62] Kleppner SR, Robinson KA, Trojanowski JQ, Lee VM-Y. Transplanted human neurons derived from a teratocarcinoma cell line (NTera-2) mature, integrate, and survive for over 1 year in the nude mouse brain. J Comp Neurol 1995;357:618-632.

[63] Morassutti DJ, Staines WA, Magnuson DSK, Marshall KC, McBurney MW. Murine embryonal carcinoma-derived neurons survive and mature following transplantation into adult rat striatum. Neuroscience 1994;58:753-763.

[64] Wojcik BE, Nothias F, Lazar M, Jouin H, Nicolas J, Peschanski M. Catecholaminergic neurons result from the intracerebral implantation of embryonal carcinoma cells. Proc Natl Acad Sci USA 1993;90:1305-1309.

[65] Miyazono M, Lee VM-Y, Trojanowski JQ. Proliferation, cell death, and neuronal differentiation in transplanted human embryonal carcinoma (NTera2) cells depend on the graft site in nude and severe combined immunodeficient mice. Lab Invest 1995;73:273-283.

[66] Isacson O, Deacon TW. Specific axon guidance factors persist in the mature rat brain: evidence from fetal neuronal xenografts. Neuroscience 1996;75:827-837.

[67] Hemmati-Brivanlou A, Melton D. Vertebrate embryonic cells will become nerve cells unless told otherwise. Cell 1997;88:13-17.

[68] Grunz H, Tacke L. Neural differentiation of Xenopus laevis ectoderm takes place after disaggregation and delayed reaggregation without inducer. Cell Diff Dev 1989;28(211-218).

[69] Wilson PA, Hemmati-Brivanlou A. Induction of epidemis and inhibition of neural fate by Bmp-4. Nature 1995;376:331-333.

[70] Hogan B. Bone morphogenic proteins: multifunctional regulators of vertebrate development. Genes Dev 1996;10:1580-1594.

[71] Pichel J, Shen L, Sheng H, Granholm A, Drago J, Grinberg A, Lee E, Huang S, Saarma M, Hoffer B, et al. Defects in enteric innervation and kidney development in mice lacking GDNF. Nature 1996;382:73-76.

FIGURE LEGENDS

Figure 1. Target-specific innervation by grafted fetal cells. (A) Target zones in adult host gray matter are selectively innervated by embryonic pig donor DA axons normally destined to form synapses there, whereas non-DA donor fibers grow into host myelinated bundles. (B) In the intact rat, the subpopulation of nigral DA neurons from A9 SNc, which co-express AHD, project their axons to the gray matter of dorsal-lateral regions of the striatum. The ventral tegmental area (VTA)

neurons from A10 co-express CCK, and project to ventromedial striatum, nucleus accumbens, neocortex and limbic regions. (C) When the enriched population of TH/AHD neurons obtained from a medial (versus lateral) VM dissection is transplanted into DA-lesioned adult rat striatum, these neurons preferentially reinnervate their normal dorsolateral striatal target, shown to be involved in rotational recovery after DA neuron transplantation. TH/CCK neurons from VM show different patterns of outgrowth when placed into cortex. (Reprinted with permission from Trends in Neurosciences 1997; 20:477-482. © Elsevier.)

Figure 2. Basic steps for ES cell procedures including in vitro expansion, chemical or spontaneous induction into neurons after implantation into the adult brain. Totipotent embryonic stem cells derived from the inner cell mast of blastocyst are propagated in culture in the presence of leukemia inhibitory factor (LIF). Prior to transplantation, LIF is removed. The cells are treated with retinoic acid (A) or are transplanted directly (B) into adult brain. Regardless of pre-treatment with retinoic acid, the transplanted ES cells differentiate to form cells with neuron-like morphology and phenotypic expression of neuronal markers.

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Cell and Synaptic Replacement Therapy for Parkinson's Disease: Current Status and Future Directions

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Parkinson's disease (PD) is caused by a degeneration of dopaminergic (DA) neurons located in the ventral mesencephalon. Cell replacement therapy seeks to replace the loss in synaptic signaling cause by the neuronal degeneration. It has been shown that fetal ventral mesencephalic neurons transplanted to the caudate/putamen of PD patients can significantly reduce the need of L-dopa treatment and improve symptoms. However, the fetal cell grafting procedure is limited to a few centers worldwide and will not become a standard treatment until some major issues are solved. The current major problem is the use of fetal tissue that raises ethical concerns and is impracticable since tissue from several fetuses is needed due to low neuronal survival after grafting. It is also of importance to achieve increased axonal outgrowth and synaptic reinnervation from the grafted cells. Recent findings in stem cell research have indicated that stem cells might be a very potential cell donor source for cell replacement therapy. In addition, new insights into axon guidance mechanisms will provide tools for stimulating outgrowth and achieving appropriate target innervation from the grafted cells. This review discusses the development of cell replacement therapy for PD and also looks forwards towards future possibilities on how to increase the efficacy and availability of this treatment.

Keywords: Dopamine • transplantation • L-dopa • stem cells • axon guidance

Patients presently with Parkinson's disease (PD) can only benefit from pharmacological treatment for about 5-10 years due to a continued loss of dopamine (DA) neurons and synapses, a gradual increase in side effects and decreased sensitivity to the drugs. The major cause of Parkinson's disease is a selective loss of DA neurons in the ventral mesencephalon, and one obvious way to seek a cure has to been to try to substitute the lost DA neurons with implantation of new cells producing adequate dopamine.

The current problems and opportunities in neural repair by implanted cells revolve around how to achieve a practical, reliable and reproducible source of donor cells that survive and provide new functional synapses in appropriate areas of the brain and spinal cord. To date, most technical efforts have been focused

on increasing survival of grafted DA neurons. However, to achieve sufficient functional effects by the grafted cells, not only do the cells need to survive in the new location, but must also form synaptic contacts in the patient's brain.

In this review we discuss the current status of cell replacement therapy for PD and future directions for achieving a reproducible effective therapy for PD.

TRANSPLANTATION OF NEURAL TISSUE TO THE CENTRAL NERVOUS SYSTEM

The first report of apparently successful brain grafting came from Elisabeth Dunn in 1917 [1]. In her experiment, neonatal rat cortical tissue that had been grafted to the cortex of rat pups retained many morphological features. This report showed the importance of using developing rather than mature donor material for grafting. Further evidence for this came in 1940 when W. E. LeGros Clarke reported successful grafting of fetal cortical tissue into the neonatal brain [2]. Interestingly, functional effects were obtained by K. M. Knigge in 1962 [3] and in 1963 by B. Halasz *et al.* [4], who showed that neonatal pituitary tissue grafted to the third ventricle could restore growth and reproductive function in pituitary ectomized rats.

Sixteen years later, Perlow *et al.* [5], as well as Stenevi and Björklund [6] (1979) were able to show that grafts of fetal ventral mesencephalic DA neurons could compensate for the DA motor deficits produced by a unilateral 6-OHDA lesion in the rat. In the years following this report, the scientific community was able to produce extensive proof that grafts of fetal DA neurons could survive the grafting procedure for a long time period, have spontaneous electrical activity [7], and establish both afferent and efferent synaptic contacts with the host brain [8]. This form of cell transplantation produced symptomatic relief in various animal models of PD. Based on this extensive and encouraging work in animal models, in 1987, a research team from Lund, Sweden lead by Lindvall and colleagues performed the first grafting of fetal human ventral mesencephalic tissue to a patient with PD [9]. Inspired by the pioneering work by the Lund group, other research teams soon followed and currently, many groups worldwide are now using DA transplants as an experimental therapy.

CURRENT AND POTENTIAL THERAPEUTIC BENEFITS FROM NEURONAL CELL IMPLANTATION IN PARKINSON'S DISEASE

The development of fetal neural transplantation for PD has shown (when appropriate methods are used for transplantation) that most patients show an improvement and reduction in drug-induced dyskinesias and dystonias in the "ON" phase, as well as percent time spent in "OFF" [10]. These findings are highly correlated with the presence of surviving dopaminergic grafts. In the best cases reported so far, patients have reduced their L-dopa usage to zero, in association with 50-80% reductions in prior symptoms as assessed by the clinical rating scale (*e.g.* UPDRS). The fact, that the current clinical groups are using different techniques and with different success rates [10] indicates that neural DA transplantation is still experimental and in an early technical phase. Thus, the skepticism levied at some of their results and inconsistencies is understandable. One of the most reliable findings after transplantation is some reduction of the use of L-dopa a few months after implantation and a dramatic loss of dyskinesias and "ON/OFF" phenomena with full L-dopa dose. The most likely explanation for this finding is that the biosynthetic machinery provided by the implanted new cells allows L-dopa, through dopamine decarboxylase and other synaptic vesicular transport mechanisms; to be converted to dopamine, that is released and regulated at the synaptic sites in the host brain. This interpretation of neural transplantation effects in PD also provides an understanding of the progressive improvements seen. The patients who no longer need L-dopa after transplantation all demonstrated a progressive improvement over a six-month to six-year period that is sustained [10, 11]. Moreover, the degeneration seen of the *host* dopaminergic systems also continues with no apparent effect on the implanted cells. This becomes apparent when analyzing data from unilaterally transplanted patients [12]. In Lindvall's first patients (number 3 and 4), as the grafted side became functional, the contralateral side continued to degenerate [10]. In this way, a form of hemiparkinsonism developed and the patients were relieved by subsequent transplantation bilaterally [10]. This type of clinical case-by-case evidence is complemented by a solid base of research in rodents and monkeys that demonstrates that the CNS dopaminergic system can be repaired [13]. Thus it is evident that the fetal cells that grow into the host brain are capable of taking over the function of the degenerated host systems.

CURRENT PROBLEMS IN ESTABLISHING NEURONAL CELL IMPLANTATION AS A TREATMENT FOR PARKINSON'S DISEASE

Sources of Reliable Donor Cells

Although grafting of fetal DA cells to PD patients shows great promise, several current limitations have to be overcome before this methodology can become a standard treatment for PD. One such problem at the present time is that fetal tissue for one PD patient has to be taken from about 4-8 aborted fetuses, which is

ethically controversial and restricts the use of the method to Research Centers with a high turnover of abortions at early gestations. Some authors have argued [14] based on postmortem studies of grafted PD patients, [15] that about 80,000 surviving DA neurons per striatum are needed to achieve symptomatic relief, although the reasoning for this assumes that all dopamine neurons are functionally equal (while A10 and A9 DA neurons are not). The major reason for the large amount of tissue needed per patient has been the relatively low survival of the grafted fetal neurons after transplantation. Unfortunately, only 5-10% of the grafted DA cells have been able to survive the transplantation procedures. Great efforts have been made to try to solve the problem of poor post transplantation viability. Using animal models, increased DA neuronal survival has been reported using growth factors like glial cell line-derived neurotrophic factor (GDNF) [16]. Similar results have been reported using antioxidative agents like lazaroids [17, 18]. However, independently of which agent was used, a survival plateau of around 30-40% of the implanted cells was reached. Since the ventral mesencephalon of a human fetus contains about 1×10^6 neurons and approximately 10% of these are DA neurons, 2-3 fetuses per side would still be needed to produce enough surviving DA neurons even with maximized trophic support.

Due to the many problems connected to the use of fetal DA neurons, many scientists have now began the search for an alternative source to produce functional DA neurons. Fetal pig DA neurons have been tried as an alternative source and these neurons can survive and reinnervate the host brain when grafted to PD patients [19]. However, the survival of the pig neurons has been poor and there are also infectious and immunological issues that have to be considered using xenografting techniques as a standard treatment.

The Use of Stem Cells

A research direction that has attracted a lot of attention and that seems very exciting and promising is the use of stem cells. In its broadest definition, a stem cell is a self-renewing cell that can give rise to multiple mature phenotypes. This includes a wide variety of immature cell types from all stages of embryogenesis as well as certain developmentally sequestered cell populations in organs and tissues of the adult, such as hematopoietic stem cells and cells within the forebrain subventricular zone. While it is used interchangeably with stem cell, the term "a progenitor cell" usually represents a cell with more restricted potential to differentiate (see Johe, this volume). Many laboratories have reported that neural progenitor cells can be isolated from the developing embryonic brain as well as from the adult brain and spinal cord [20-23]. These multipotential stem cells can be expanded *in vitro* in the presence of mitogens such as fibroblast growth factor-2 (FGF-2) or epidermal growth factor (EGF) and upon withdrawal of mitogens differentiate into all three major cell types of the CNS namely, neurons, astrocytes, and oligodendrocytes. Notably, individual extracellular signals have been shown to induce these multipotential cells to alternative differentiation pathways. Thus, neural stem cells efficiently differentiate into astrocytes in the presence of ciliary neurotrophic factor (CNTF) [24]. Bone morphogenetic proteins (BMPs) have also been shown to facilitate differentiation of progenitor cells to astrocytes [25]. There is a tremendous difference in this self-renewing capacity of stem cells to differentiate into distinct cell types, which corresponds to the progressive restriction of potential that occurs during development. Recent research has focused on the derivation, characterization, and control of proliferation and differentiation of pleuripotent cells capable of generating multiple CNS cellular phenotypes.

In order to develop viable transplantation techniques using such putative neural precursors, one needs to isolate and expand the selected cells. Pleuripotent progenitor cells derived from fetal or adult brains can be expanded *in vitro,* treated with certain signaling molecule(s), and/or genetically modified for optimal differentiation to therapeutically relevant neural phenotypes. Recent progress on molecular mechanisms underlying differentiation and neurotransmitter specification of DA neurons provides an unprecedented opportunity for cell-based therapy of PD and other neurodegenerative diseases (Figure 1). Transcriptional control mechanisms for neuronal differentiation and neurotransmitter identity have been identified in organisms such as C. elegans, Drosophila, and mouse [26]. For example, a new orphan member of the nuclear receptor superfamily, Nurr1, was isolated and found to be co-expressed with TH in CNS DA neurons such as substantia nigra and olfactory bulb [27, 28]. Recent studies of Nurr1 knock out mice showed that Nurr1 is essential for the later stages of DA cell development by inducing a DA phenotype in the substantia nigra [29]. These studies suggest that Nurr1 regulates the DA phenotype directly or indirectly, although, at present, the direct target gene(s) of Nurr1 are not defined. Genetic manipulation of stem cells using recent discoveries regarding key transcription factors in DA neuronal differentiation may provide a very useful way of achieve large amounts of DA cells for transplantation [30] (Figure 1).

Increasing the DA Synaptic Reinnervation Capacity of the Grafted Cells

It is necessary to achieve survival of grafted DA neurons, but it may not be sufficient to obtain an optimal

COMPLEMENTARY PATTERNS OF EPHRIN B2 AND EPH B1 RECEPTOR IN THE DEVELOPING NIGROSTRIATAL CIRCUIT

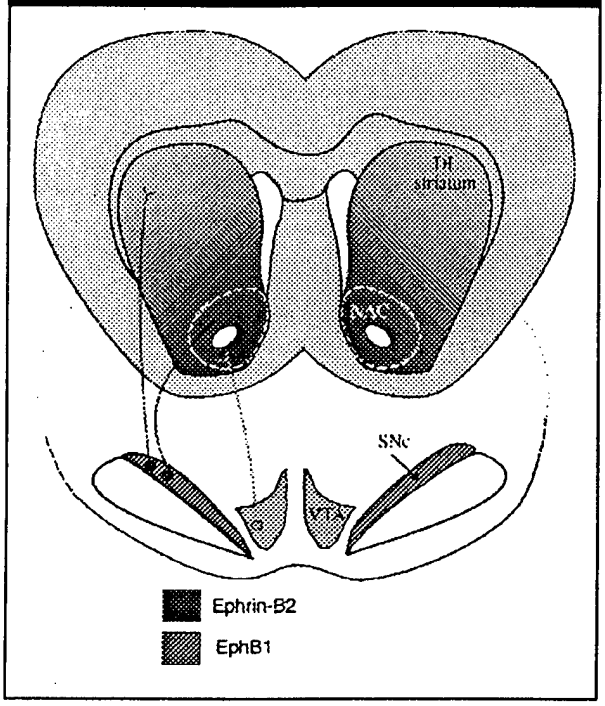

FIGURE 2
Schematic drawing showing the striatal expression pattern of Ephrin B2 (red) and the ventral mesencephalic distribution of the Eph B1 receptor (blue) in the developing rat brain. Ephrin B2 is expression is expressed a gradient with high levels in the nucleus accumbens and ventro-medial striatum, while levels are low in the dorsolateral striatum. The corresponding receptor Eph B1 is exclusively expressed in the substantia nigra pars compacta (SNc) and not the ventral tegmental area (VTA) neurons. The high levels of the Ephrin B2 ligand in nucleus accumbens and ventro-medial striatum will prevent the Eph B1 expressing growth cones from SNc DA neurons from entering this area since the interaction between ligand and receptor induces growth cone collapse. This interaction does not occur in the dorso-lateral striatum thus allowing the SNc DA neurons to innervate this area. (This figure is modified from Yue *et al.*, 1999.)

therapeutic effect. It is very important that the grafted neurons establish appropriate synaptic contact with the target territory [13]. Some efforts have been made to try to stimulate increased axonal outgrowth and branching from the surviving grafted DA neurons. Both intrinsic and extrinsic mechanisms of increased fiber outgrowth have been investigated [31]. It has been shown that transgenic overexpression of the anti-apoptotic factor Bcl-2 can increase axonal outgrowth from grafted fetal DA neurons [32, 33]. Xenografts of fetal pig DA neurons that normally develop over a longer time period than rat DA neurons will also grow for longer distances to appropriate targets in rat hosts, further indicating the fact that intrinsic factors play an important role in axonal outgrowth [13, 34]. Extrinsic factors shown to increase axonal outgrowth capacity from fetal grafted DA neurons are GDNF [35] and immunophilin ligands [36]. These factors are now seriously considered for inclusion in clinical transplantation trials.

DEVELOPING DA AXONS FIND THE WAY TO THEIR TARGETS BY RESPONDING TO BOTH INHIBITORY AND GROWTH PROMOTING FACTORS

Recently, several DA axon guidance molecules have been characterized. The dopaminergic neurons in the substantia nigra are dividing between E11 and E16 [37], and their axons begin reaching the striatal target around E 13.5 [38]. Recent studies of the formation of the nigrostriatal circuit, have indicated functions of axon guidance molecules of the Semaphorin [39], Netrin [40], Ephrin and the Eph receptor [41] families. The Eph receptors are the largest known family of tyrosine receptor kinases and they play important roles in cell growth, survival, migration and connectivity [42, 43]. The Eph receptors can be divided into two subfamilies; Eph A receptors (Eph A1 to Eph A8) and Eph B receptors (Eph B1 to Eph B6), corresponding to their ligand specificity. The ligands are called Ephrins and consist of two subclasses. Ephrin A1 to Ephrin A5 are linked to the cell membrane via a glycosyl-phosphotidyl inositol (GPI) linkage while the Ephrin B1 to Ephrin B3 are transmembrane proteins. So far, one ligand (Ephrin B2) and one receptor (Eph B1) have been shown to be expressed in a gradient pattern within the nigrostriatal circuit [41] (Figure 2). When an Eph B1 receptor on a growth cone binds to the Ephrin B2 ligand, this will cause growth cone collapse and also send apoptosis inducing signals to the neuron, thus repelling the axon. During development of the nigrostriatal circuit, the Eph B1 receptor is expressed only in the DA neurons of the substantia nigra pars compacta (SNc) and not in ventral tegmental area (VTA) DA neurons while the corresponding ligand Ephrin B2 is expressed in a gradient pattern within the target striatum. High levels of Ephrin B2 can be found in the ventromedial striatum and nucleus accumbens while levels are low in the dorsolateral striatum (Figure 2). This gradient restricts the SNc DA neurons from growing into the ventromedial striatum and nucleus accum-

bens, but they will not be repelled from the dorsolateral striatum (their appropriate synaptic target area). These findings by Yue and collaborators [41] have started to reveal some of the mechanisms involved in how developing DA neurons find their target areas. Further knowledge about the axon guidance mechanism can allow manipulations to increase the synaptic reinnervation capacity of grafted DA cells.

In conclusion, from fairly inauspicious beginnings, DA fetal and stem cell neural transplantation is providing a completely new approach for treatment of PD. Rational pharmacological replacement of the dead DA neurons in PD by L-dopa, while initially helpful, eventually becomes insufficient with debilitating side-effects for the patient. Since the loss of efficacy is likely due to loss of L-dopa conversion and DA synaptic control (homeostatic) mechanisms of transmitter release, reinnervation with new synapses by implanted DA neurons can potentially provide a better intervention. Neural DA cell replacement for PD requires better techniques and donor cells to become a standard and reliable therapy. Hopefully, scientific insights about new donor cell sources, their axonal and synaptic integration in the patients brain as well as technical development in neurosurgery (see Mendez *et al.*, this volume) will achieve a translation of these novel ideas into clinical practice.

ACKNOWLEDGEMENTS

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REFERENCES

1. Dunn E. Primary and secondary findings in a series of attempts to transplant cerebral cortex in the albino rat. J Comp Neurol 1917;27:565-82.
2. LeGros Clarke WG. Neuronal differentiation in implanted fetal cortical tissue. J Neurol Psychiatry 1940;3:263-84.
3. Knigge KM. Gonadotrophic action of neonatal pituitary glands implanted in the rat brain. Amer J Physiol 1962;202:387-91.
4. Halasz B, Pupp L, Uhlarik S, Tima L. Growth of hypophysectomised rats bearing pituitary transplants in the hypothalamus. Acta Physiologica Scientifica Hungaria 1963;23:287-92.
5. Perlow M, Freed W, Hoffer B, Seiger A, Olson L, Wyatt R. Brain grafts reduce motor abnormalities produced by destruction of nigrostriatal dopamine system. Science 1979;204:643-7.
6. Bjorklund A, Stenevi U. Reconstruction of the nigrostriatal dopamine pathway by intracerebral nigral transplants. Brain Res 1979;177:555-60.
7. van Horne CG, Mahalik T, Hoffer B, Bygdeman M, Almquist P, Stieg P, *et al.* Behavioral and electrophysiological correlation of human mesencephalic dopaminergic xenograft function in the rat striatum. Brain Res Bulletin 1990;25:325-34.
8. Freund TF, Bolam JP, Bjorklund A, Stenevi U, Dunnett SB, Powell JF, *et al.* Efferent synaptic connections of grafted dopaminergic neurons reinnervating the host neostriatum. J Neurosci 1985;5:603-16.
9. Lindvall O, Rehncrona S, Gustaavii B, Brundin P, Åstedt B, Widner H, *et al.* Fetal dopamine-rich mesencephalic grafts in Parkinson's disease. Lancet 1988;2:1483-4.
10. Lindvall O. Update on fetal transplantation: the Swedish experience. Mov Disorders 1998;13:83-7.
11. Mendez I, Dagher A, Hong M, Hebb A, Gauder P, Law A, *et al.* Enhancement of survival of stored dopminergic cells and promotion of graft survival by exposure of human fetal nigral tissue to glial cell line—derived neurotrophic factor in patients with Parkinson's disease. J Neurosurg 2000;92:863-9.
12. Piccini P, Brooks DJ, Bjorklund A, Gunn RN, Grasby PM, Rimoldi O, *et al.* Dopamine release from nigral transplants visualized *in vivo* in a Parkinson's patient. Nature Neurosci 1999;2:1047-8.
13. Isacson O, Deacon TW. Neural transplantation studies reveal the brain's capacity for continuous reconstruction. Trends Neurosci 1997;20:477-82.
14. Bjorklund A, Lindvall O. Cell replacement therapies for central nervous system disorders. Nature Neurosci 2000;3:537-44.
15. Kordower JH, Freeman TB, Snow BJ, Vingerhoets FJG, Mufson EJ, Sanberg PR, *et al.* Neuropathological evidence of graft survival and striatal reinnervation after the transplantation of fetal mesencephalic tissue in a patient with Parkinson's disease. New Engl J Med 1995;332:1118-24.
16. Granholm AC, Mott JL, Bowenkamp K, Eken S, Henry S, Hoffer BJ, *et al.* Glial cell line-derived neurotrophic factor improves survival of ventral mesencephalic grafts to the 6-hydroxydopamine lesioned striatum. Exp Brain Res 1997;116:29-38.
17. Nakao N, Frodl E, Duan WM, Widner H, Brundin P. Lazaroids improve the survival of grafted embryonic dopamine neurons. Proc Natl Acad Sci 1994;91:12408-12.
18. Bjorklund L, Spenger C, Stromberg I. Tirilazad mesylate increases dopaminergic neuronal survival in the in oculo grafting model. Exp Neurol 1997;148:324-33.
19. Deacon T, Schumacher J, Dinsmore J, Thomas C, Palmer P, Kott S, *et al.* Histological evidence of fetal pig neural cell survival after transplantation into a patient with Parkinson's disease. Nature Med 1997;130:350-3.
20. Brustle O, McKay RD. Neuronal progenitors as tools for cell replacement in the nervous system. Curr Opin Neurobiol 1996;6:688-95.
21. Gage FH. Mammalian neural stem cells. Science 2000;287:1433-8.
22. Gritti A, Frolichsthal-Schoeller P, Galli R, Parati EA, Cova L, Pagano SF, *et al.* Epidermal and fibroblast growth factors behave as mitogenic regulators for a single multipotent stem cell-like population from the subventricular region of the adult mouse forebrain. J Neurosci 1999;19:3287-97.
23. Isacson O. The neurobiology and neurogenetics of stem cells. Brain Pathol 1999;9:495-8.
24. Johe KK, Hazel TG, Muller T, Dugich-Djordjevic MM, McKay RD, Vicario-Abejon C, *et al.* Single factors direct the differentiation of stem cells from the fetal and adult central nervous system. Functions of basic fibroblast growth factor and neurotrophins in the differentiation of hippocampal neurons. Genes Dev 1996;10:3129-40.

25. Gross RE, Mehler MF, Mabie PC, Zang Z, Santschi L, Kessler JA. Bone morphogenetic proteins promote astroglial lineage commitment by mammalian subventricular zone progenitor cells. Neuron 1996;17:595-606.
26. Goridis C, Brunet, J-F. Transcriptional control of neurotransmitter phenotype. Curr Opinion Neurobiol 1999;9:47-53.
27. Law SW, Conneely OM, DeMayo FJ, O'Malley BW. Identification of a new brain-specific transcription factor, Nurr1. Mol Endocrinol 1992;6:2129-35.
28. Zetterstrom RH, Williams R, Perlmann T, Olson L. Cellular expression of the immediate early transcription factors Nurr1 and NGFI-B suggests a gene regulatory role in several brain regions including the nigrostriatal dopamine system. Mol Brain Res 1996;41:111-20.
29. Zetterstrom RH, Solomin L, Jansson L, Hoffer BJ, Olson L, Perlmann T. Dopamine neuron agenesis in Nurr1-deficient mice. Science 1997;276:248-50.
30. Song J, Bjorklund L, Chung S, Andersson T, Costantini L, Kim K, *et al.* Establishing Nurr1-expressing ES cell lines for neural transplantation. Soc Neurosci (abstract 2000)
31. Holm K, Isacson O. Factors intrinsic to the neuron can induce and maintain its ability to promote axonal outgrowth: a role for BCL2? Trends Neurosci 1999;22:269-73.
32. Holm KH, Boonman Z, Tandon P, Costantini LC, Deacon TW, Chen DF, *et al.* Specific graft-host synaptic connections and long distance axonal growth in fetal neurtransplantation to adult CNS. Soc Neurosci (abstract, 1998).
33. Schierle GS, Leist M, Martinou JC, Widner H, Nicotera P, Brundin P. Differential effects of Bcl-2 overexpression on fibre outgrowth and survival of embryonic dopaminergic neurons in intracerebral transplants. Eur J Neurosci 1999;9:3073-81.
34. Isacson O, Deacon TW, Pakzaban P, Galpern WR, Dinsmore J, Burns LH. Transplanted xenogeneic neural cells in neurodegenerative disease models exhibit remarkable axonal target specificity and distinct growth patterns of glial and axonal fibres. Nature Med 1995;1:1189-94.
35. Wang Y, Tien L, Lapchak P, Hoffer B. GDNF triggers fiber outgrowth of fetal ventral mesencephalic grafts from nigra to striatum in 6-OHDA-lesioned rats. Cell Tissue Res 1996;286:225-33.
36. Costantini LC, Isacson O. Immunophilin ligands and GDNF enhance neurite branching or elongation from developing dopamine neurons in culture. Exp Neurol 2000;164:60-70.
37. Lauder J, Bloom F. Ontogeny of monoamine neurons in the locus coeruleus, raphe nuclei and substantia nigra of the rat. J Comp Neurol 1974;155:469-82.
38. Olson L, Seiger A. Early prenatal ontogeny of central monomine neurons in the rat: Fluorescens histochemical observations. Z Anat Entwickl.-Gesch 1972;137:301-16.
39. Skaliora I, Singer W, Betz H, Püschel AW. Differential patterns of semaphorin expression in the developing rat brain. Eur J Neurosci 1998;10:1215-29.
40. Livesey FJ, Hunt SP. Netrin and Netrin receptor expression in the embryonic mammalian nervous system suggests roles in retinal, striatal, nigral and cerebellar development. Molec Cellular Neurosci 1997;8:417-29.
41. Yue Y, Widmer DAJ, Halladay AK, Cerretti DP, Wagner GC, Dreyer J-L, *et al.* Specification of distinct dopaminergic neural pathways: roles of the Eph family receptor EphB1 and ligand Ephrin-B2. J Neurosci 1999;19:2090-101.
42. Gale NW, Yancopoulos GD. Ephrins and their recceptors: a repulsive topic? Cell Tissue Res 1997;290:227-41.
43. Zhou R. The Eph family receptors and ligands. Pharmacol Ther 1998;77:151-81.

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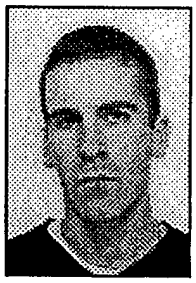

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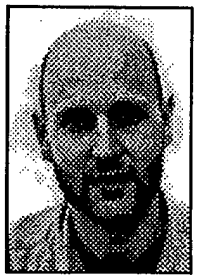

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LONG-TERM IN VIVO PET/MRS NEURODEGENERATION STUDIES OF A PRIMATE PARKINSON'S DISEASE MODEL.

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Parkinson's disease (PD) signs were induced in Cynomolgus monkeys using long-term low-dose MPTP injections (over 14 +/- 5 months) until a full PD syndrome appeared. Serial PET studies were carried out using (C-11) CFT as a ligand for pre-synaptic dopamine reuptake sites (DARS). Proton MRS studies of striatal neurochemicals: choline (Cho), creatine (Cr), N-acetylaspartate (NAA) and lactate were also performed. The binding potential of (C-11) CFT on DARS gradually declined after MPTP-administration, faster in putamen than in caudate, and were reduced to 12 +/- 2% of the corresponding control value in normal putamen by 5-8 months after termination of MPTP and remained at those levels until the end of 2 years follow up period. DARS in caudate were reduced to 21 +/- 9% 5-8 months after MPTP and remained reduced to 18 +/- 4% 2 years post MPTP, indicating that functional degeneration of dopamine terminals with sites for dopamine transporter was not spontaneously reversible. Parallel MRS studies showed an elevation of lactate and choline peaks. Ten months post MPTP the observed striatal elevation in the lactate peak was considerable, yet at 2 years post MPTP the values returned to control levels. The Cho/Cr ratio in control animals was 0.83 +/- .06 while it was 1.30 +/- .15 in the time period of 8-10 months after MPTP. NAA/Cr ratio was stable in control studies (2.38 +/- .11). This ratio decreased slightly in MPTP-treated monkeys being 2.09 +/- .29 in the striatum 8-10 months after MPTP. Two years after MPTP the observed increase of choline was 38 +/- 4% of the control value, and the corresponding decrease of NAA was 26 +/- 4% indicating irreversible changes.

These combined PET/MRS studies in a PD model demonstrate that imaging of specific receptor sites associated with PD, as well as changes in neurochemicals, can be used as a sensitive indicator for progression of neurodegeneration.

(Supported by NINDS and Massachusetts General Hospital.)

Key Words: (see instructions p. 4)

1. Parkinson's disease
2. MPTP
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4. Magnetic Resonance Imaging

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The signing member certifies that any work with **human or animal subjects** related in this abstract complies with the guiding policies and principles for experimental procedures endorsed by the Society. This signature acknowledges that each author on this abstract has seen and approved the final version of the abstract and has given consent to appear as an author. Abstracts must comply with ethical guidelines for human and animal research, and authors may be asked to supply added documentation.

Ole Isacson — Printed or typed name

(617) 855-3283 — Telephone number

Society for Neuroscience member's signature

SOCIETY FOR NEUROSC
1999 ABSTRACT FO

Dr. Ole Isacson
Publication 7
DAMD17-98-1-8618

Read all instructions before typing.
See *Call for Abstracts* and reverse of this sheet.
Complete abstract and **all boxes at left and below** before making copy
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each replacement abstract. Replacement abstracts must be **RECEIVED** by Wednesday, May 5, 1999.

SMALLEST RECOMMENDED TYPE SIZE: 10 POINT

SAMPLE:
1999 Annual Meeting
Miami Beach, Florida
October 23–28, 1999

POSTMARK DEADLINE:

MONDAY, APRIL 26, 1999

First (Presenting) Author

Provide full name (no initials), address, and phone numbers *of first author on abstract. You may present (first author) only one abstract.* (Please type or print in black ink.)

Anna-Liisa Brownell
Department of Radiology
Bartlett Hall Ext. 500 R
Massachusetts General Hospital
Boston, MA 02114 Fax: (617) 726-5123
Office: (617) 726-3807 Home: (617) 744-3725
E-mail: abrownell@partners.org

An asterisk must be placed after the sponsor's (signing member) name on the abstract.

The present scientific work may involve a real or perceived financial conflict of interest. (See page 4, item 11.)

☐ yes ☐ no If yes:

Presentation Preference

Check one: ☒ poster ☐ slide

Themes and Topics

See list of themes and topics, pp. 17–18. Indicate below a first and second choice appropriate for programming and publishing your paper.

1st theme title: Neurotransmitters Modulators, Tran theme letter: D
1st topic title: Other neuro-transmitters topic number: 57

2nd theme title: Neurotransmitters Modulators, Tra theme letter: D
2nd topic title: Uptake and Transporters topic number: 60

Special Requests (for example, projection, video, or computer requirements)

Include nonrefundable **abstract handling fee of $40.** Fill out payment information form below. Purchase orders will not be accepted. Submission of abstract handling fee does not include registration for the Annual Meeting.

PET and phMRI studies of dopamine receptor modulation in PD models.

A-L. Brownell*[1,2], T. van Nguyen[2], Y-C. J. Chen[2], F. Cavagna[2], B.R. Rosen[2], O. Jsacson[1], B.Q. Jenkins[2]. [1]Neuroregeneration Laboratory, McLean Hospital, Belmont, MA 02178. [2]Department of Radiology, Massachusetts General Hospital, Boston, MA 02114.

In PD, degeneration of dopamine synapses and associated loss of reuptake sites and dopamine could be the reason for enhanced binding to dopamine D_2 receptors. To examine the coupling between dopamine receptors and transmitters we conducted PET and phMRI studies in 6-OHDA lesioned rats. The binding ratio of ^{11}C-CFT reduced in the lesioned striatum 65-85 % compared to the intact side indicating depletion of dopamine reuptake sites. In the same area the binding ratio of ^{11}C-raclopride was increased 19-25 % indicating upregulated binding of dopamine D_2 receptors. The same area showed 3-6 % decrease in 11C-SCH binding indicating light decrease in dopamine D_1 receptor binding.

The concept of supersensitivity has been thoroughly investigated in the dopamine system in PD and related animal models. It has been shown in 6-OHDA rat model that cerebral blood flow and striatal D_2 receptor binding are increased on the lesioned side after apomorphine stimulation. We used functional MRI with pharmacological stimulation with amphetamine (3mg/kg i.v.) and apomorphine (2 mg/kg i.v.) and observed significantly increased signal change indicating increased regional blood volume in striatum, frontal cortex and thalamus in lesioned side after apomorphine stimulation. With amphetamine stimulation these same brain areas showed enhanced signal in intact side.

Key Words: (see instructions p. 4)

1. dopamine receptor
2. blood flow
3. PET
4. MRI

Signature of Society for Neuroscience member required below. ***No member may sign more than one abstract. The signing member must be an author on the paper and an asterisk must be placed after the sponsor's (signing member) name on the abstract.***

The signing member certifies that any work with **human or animal subjects** related in this abstract complies with the guiding policies and principles for experimental procedures endorsed by the Society. This signature acknowledges that each author on this abstract has seen and approved the final version of the abstract and has given consent to appear as an author. Authors must comply with ethical guidelines for human and animal research, and may be asked to supply added documentation.

Anna-Liisa Brownell — Society for Neuroscience member's signature

Anna-Liisa Brownell — Printed or typed name

(617) 726-3807 — Telephone number

COMPLEMENTARY PET STUDIES OF STRIATAL DOPAMINERGIC SYSTEM AND CEREBRAL METABOLISM IN A PRIMATE MODEL OF PARKINSON'S DISEASE.

A-L. Brownell*[1,2], Y-I. Chen[1], E. Livni[1], F. Cicchetti[2], O. Isacson[2]. [1]Department of Radiology, Massachusetts General Hospital, Boston, MA 02114. [2]Neuroregeneration Laboratory, McLean Hospital, Belmont, MA 02478.

Degeneration of dopamine (DA) synapses in Parkinson's disease (PD) is associated with the loss of striatal dopamine release and dopamine reuptake sites. To compensate for this loss of innervation, the remaining postsynaptic DA targets (D_2 receptors) might have enhanced capacity to bind dopamine.

To assess the relationship between dopamine transporters, D_2 receptors and energy metabolism we conducted PET imaging studies in a primate MPTP model of PD. PET imaging studies were conducted before and 2 and 3 months after the last MPTP administration. The binding ratio of ^{11}C-CFT to the striatal dopamine transporters was reduced by 65-72 % indicating loss of dopamine transporter sites. In the same areas the binding ratio of ^{11}C-raclopride was increased 18-22% indicating significantly enhanced binding to the postsynaptic dopamine D_2 receptors. Studies of energy metabolism conducted with ^{18}F-2-fluorodeoxy-D-glucose showed only minor increases in glucose utilization in the same striatal areas.

These data indicate that decrease of striatal dopamine release, induced by depletion of presynaptic dopamine reuptake sites of up to 72%, enhances binding of dopamine to postsynaptic D_2 receptors. Minor increases in total PET glucose utilization was observed in such striatal areas, possibly reflecting a maintained neuronal function at this level of DA loss or evidence of degenerative processes.

2000 ABSTRACT FORM

Check here if this is a

Read all instructions befor
See *Call for Abstracts* and re
Complete abstract and **all box**
(Please type or print i

Dr. Ole Isacson
Publication 9
DAMD17-98-1-8618

First (Presenting) Author

Provide full name (no initials), address, and phone numbers *of first author on abstract. You may present (first author) only one abstract.* (Please type or print in black ink.)

Dr. Francesca Cicchetti
Neuroregeneration Laboratories
McLean Hospital, MRC I
115 Mill St. Belmont
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Office: (617) 855-2381 Home: (617) 924-8054
E-mail: fcicchetti@mclean.harvard.edu

SMALLEST RECOMMENDED TYPE SIZE: 10 POINT

SAMPLE:
2000 Annual Meeting
New Orleans, La.
November 4–9, 2000

POSTMARK DEADLINE:

MONDAY, APRIL 24, 2000

An asterisk must be placed after the sponsor's (signing member) name on the abstract.

MANDATORY: The present scientific work may involve a real or perceived financial conflict of interest. (See page 4, item 11.)
☐ yes ☒ no If yes: _____

Presentation Preference

Check one: ☒ poster only ☐ slide/poster

Themes and Topics

See list of themes and topics, pp. 17–18. Indicate below a first and second choice appropriate for programming and publishing your paper.

1st theme title: Development and Regeneration **theme letter:** A
1st topic title: Neuronal death **topic number:** 17

2nd theme title: Disorders of the neurons... **theme letter:** J
2nd topic title: Degenerative disease Parkin **topic number:** 131

Special Requests (for example, projection, video, or computer requirements)

Include nonrefundable **abstract handling fee** of **$50.** Fill out payment information form below. Purchase orders will not be accepted. Submission of abstract handling fee does not include registration for the Annual Meeting.

NEUROINFLAMMATION OF THE NIGRO-STRIATAL PATHWAY DURING PROGRESSIVE DOPAMINE DEGENERATION.

F. Cicchetti*[1], A.L. Brownell[1,2], Y.I. Chen[2], E. Livni[2] & O. Isacson[1].
[1]Neuroregeneration Laboratory, McLean Hospital/Harvard Medical School, Belmont, MA 02478; [2]Department of Radiology, Massachusetts General Hospital/Harvard Medical School, Boston, MA 02114.

We investigated the inflammatory response in vivo during dopamine degeneration using PET imaging of dopamine transporters and microglial activation in a rat model of progressive dopamine neuron degeneration induced by unilateral (right) intrastriatal administration of 6-hydroxydopamine (6-OHDA). Intrastriatally administrated 6-OHDA produces retrograde degenerative changes in dopaminergic neurons of the substantia nigra through oxidative and mitochondrial damage and thus creates a slowly progressive degeneration of the dopaminergic system. Imaging studies of dopaminergic degeneration were conducted in 4 control rats and 5 unilaterally lesioned rats 3 weeks after 6-OHDA using ^{11}C-CFT (2ß-carbomethoxy-3ß-(4-fluorophenyl) tropane), a specific ligand for pre-synaptic dopamine transporters and ^{11}C-PK11195 (1-(2-chlorophenyl)-N-methyl-N-(1-methylpropyl)-3 isoquinoline carboxamide), a specific ligand for microglia. The dopaminergic system showed a significant decrease in binding parameters of the dopamine transporters ^{11}C-CFT in the striatum indicating degeneration of the dopaminergic system and parallel increases of ^{11}C-PK11195 binding, indicating microglial activation in the striatum and substantia nigra. These preliminary data strongly support the hypothesis that inflammation is a significant component in progressive dopaminergic degeneration.

Key Words: (see instructions p. 4)

1. imaging
2. Parkinson
3. microglia
4. Basal ganglia

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Francesca Cicchetti
Society for Neuroscience member's signature

Francesca Cicchetti
Printed or typed name

(617) 855-2381
Telephone number

000162674
Member ID number (mandatory)

Tear at perforation

HARVARD UNIVERSITY CURRICULUM VITAE

DEMOGRAPHIC INFORMATION:

Name: Ole Isacson

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McLean Hospital, MRC 119
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Belmont, MA 02478
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Home Address: 14 Ellery Square
Cambridge, MA 02138

Email: isacson@helix.mgh.harvard.edu

Place of Birth: Kristianstad, Sweden

EDUCATION AND TRAINING:

Education:

1979	Biochemistry, University-College of Kalmar, Sweden
1980	Medical School, University of Lund, Sweden
1981	Research appointment, University of Lund, in the laboratory of Prof. A. Björklund.
1983 M.B.	1st medical degree, Medical Bachelor, Medical School, University of Lund
1987 M.D.(-Ph.D.)	Doctor of Medicine (Dr. Med. Sc.), University of Lund Thesis: "Neural grafting in an animal model of Huntington's disease" (Medical Neurobiology)

Postdoctoral Training:

1987-1989	Research Fellow, Department of Anatomy (Neuroanatomy Division), University of Cambridge, England

Licensure and Certification: Not applicable.

PROFESSIONAL APPOINTMENTS:

Academic Appointments:

1981-83	Teaching Assistant Dept.of Histology, University of Lund, Sweden
1983-85	Research Assistant (Demonstrator) Dept. of Histology, University of Lund, Sweden
1986-87	Lecturer & Research Associate Dept. of Medical Cell Research, University of Lund, Sweden
1987-89	Research Fellow Dept. of Anatomy (Neuroanatomy), University of Cambridge, England

PROFESSIONAL APPOINTMENTS: (continued)

Academic Appointments: (continued)

1989-	*Docent* (Academic Title of Assoc. Professor) of Medical Neurobiology University of Lund, Sweden
1989-1992	Assistant Professor of Neurology (Neuroscience) Harvard Medical School, Boston, MA
1992-	Associate Professor of Neurology (Neuroscience) Harvard Medical School, Boston, MA

Hospital or Affiliated Institution Appointments:

1989-1996	Director of Neuroregeneration Laboratory Mailman Research Center, McLean Hospital, Belmont, MA
1989-1993	Assistant in Neuroanatomy Department of Neurology, Massachusetts General Hospital, Boston, MA
1989-	Investigator Harvard University/New England Primate Research Center, Southborough, MA
1993-	Associate in Neuroanatomy Department of Neurology, Massachusetts General Hospital, Boston, MA
1996-	Director of Neuroregeneration Laboratories (a full hospital department) Mailman Research Center, McLean Hospital, Belmont, MA

Other Professional Positions and Major Visiting Appointments:

1988-1989	CNRS and CEA Visiting Research Professor Hospital de Orsay, Paris, France
1990-	Senior Thesis Advisor, Departments of Biochemistry, Biology and Psychology Harvard University, Cambridge, MA
1990-	Postdoctoral Research Advisor for Residents in Neurology and Neurosurgery Massachusetts General Hospital, Boston, MA
1990-	Postdoctoral Research Advisor for Fellows in Neurobiology and Neurology Harvard Medical School, Boston, MA
1993-	Adjunct Associate Professor of Neuroscience University of Massachusetts Medical School, Worcester, MA
1996	Visiting Professorship Lecture Series The Miami Project ot Cure Paralysis, The University of Miami School of Medicine

AWARDS AND HONORS:

1976-78	Nathorst's Scientific Foundation. Full Scholarship Award at Atlantic College, U.K. (personal award).
1983	The Medical Faculty Award for graduate thesis work in medicine, University of Lund, Sweden (personal award).
1987	The Fernstrom Foundation Scholarship Award 1987 for medical scientists (personal prize/award).

AWARDS AND HONORS: (continued)

1987	The Swedish Physican's Society Award for studies on neurodegenerative diseases (personal prize/award).
1987	The Royal Swedish Academy of Sciences. Lindahl's Award (personal award).
1989-90	NATO Grant, for studies on neurodegenerative disease. Research grant # CRG 890583.
1990-91	NIH Program Project Award: Huntington's Disease Center, (P.I. of sub-contract) Massachusetts General Hospital and McLean Hospital.
1991-96	NIH R29 Award: Neurological Science. Research Grant # R29 NS29178.
1992-95	NIH RO1 Award: Neurological Science. Research Grant # RO1 NS30064.
1995	Milton Fund Award, Harvard University
1995-	NIH RO1 Award: Neurological Science. Research Grant # RO1 NS30064-04.
1998-02	USAMRAA Research Grant Award DAMD17-98-1-8618 (R01 level).
1999-02	USAMRAA Research Grant Award DAMD17-99-1-9482 (R01 level).
1999-	NIH: NINDS Parkinson's Research Center of Excellence. (Center Director) P50 NS39793.
1999-	The Century Foundation Research Award.
1999-	NIH RO1 Award: Neurological Science. Research Grant # RO1 NS30064-07.
2000-	NIH RO1 Award: Neurological Science. Research Grant # RO1 NS41263-01.

SERVICE ASSIGNMENTS: Not applicable.

MAJOR COMMITTEE ASSIGNMENTS:

Harvard Medical School/Affiliated Institutions

1990-1994	Graduate Admission Committee, Program of Neuroscience, Harvard Med. Sch.
1990-	Research Committee, McLean Hospital
1990-	Scientific Advisory Board, New England Regional Primate Research Center, Southborough, MA
1990-	Institutional Animal Care and Use Committee, McLean Hospital
1991-	Space Allocation Committee, McLean Hospital
1991-	Mailman Research Center Steering Committee, McLean Hospital
1993-1996	Business/Accounting Task Force, Research Subcommittee, McLean Hospital
1993-94	Search Committee for Director of Mailman Research Center, McLean Hospital
1997-	Industrial Relations Study Group, McLean Hospital
1997-	Chair of Core Imaging Facility Advisory Commmittee, McLean Hospital/Shriver Ctr.
1998-	Search Committee, Faculty for Mailman Research Center

Regional/National/International

1992-	Veterans Administration Merit Review Board
1992-	Internal Review Committee NIH Program Project grants
1993-	Special Review Committes; National Institutes of Health (NINDS): Program Projects (Site-visit teams) and Clinical Research Centers (NIH) (Ad hoc member)
1994-	Neurological Disorders Program Project Review B Committee (NINDS) (Ad hoc member)
1994-	Human Frontier Science Program (review committee)
1994-	Council, American Society for Neural Transplantation
1995-	Natural Sciences and Engineering Research Council of Canada (review committee)
1995-1996	Program Committee, American Society for Neural Transplantation (Chair)
1997-1998	President Elect, American Society for Neural Transplantation
1997	Advisory Group on Parkinson's Disease, for presentation to Veterans' Administration, the United States Congress, Washington, D.C. (Chair)
1998	The Wellcome Trust (review group)
1998-1999	President, American Society for Neural Transplantation and Repair
1998-	MDCN-2 Study Section (NIH), member
1998-	Council, International Cell Transplant Society
1999-	Program Committee, International Neural Transplantation and Repair

MAJOR ADMINISTRATIVE RESPONSIBILITIES:

1989-1996	Director	Neuroregeneration Laboratory, Mailman Research Center, McLean Hospital
1996	Director	Neuroregeneration Laboratories (a full hospital department and research program), Mailman Research Center, McLean Hospital
1996	Dept. Head	Mailman Research Center (formerly Ralph Lowell Laboratories) Research Department
1999	Center Director	NIH Udall Parkinson's Disease Research Center

PROFESSIONAL SOCIETY INVOLVEMENT:

1989-	International Brain Research Organization (IBRO) (member)
1989-	American Association for the Advancement of Science (AAAS) (member)
1989-	Society for Neuroscience (member)
1989-	European Neuroscience Association (ENA) (member)
1989-	Boston Society of Neurology and Psychiatry (member)
1989-	World Federation of Neurology Research Group on Huntington's Disease (elected member)
1993-	Huntington's Disease Society of America, Massachusetts Chapter
1994-	American Society for Neural Transplantation (ASNT) (founding member)
1994-	American Society for Neural Transplantation (Council member)
1994-	Program Committee, American Society for Neural Transplantation (Co-Chairman)
1995-	International Society of Neuropathology (member)
1995-96	American Society for Neural Transplantation (Secretary)
1995-96	Program Committee, American Society for Neural Transplantation (Chairman)
1996-	New York Academy of Science (member)
1997-	American Academy of Neurology (member)
1997	American Society for Neural Transplantation (President-elect)
1998	American Society for Neural Transplantation and Repair (President)
1998-	International Society for Cell Transplantation (Council member)
1999-	American Society of Transplantation (elected member)

COMMUNITY SERVICE (related to professional work):

Professional Consultation (Other than Patient Care)

a) Scientific and Technical Consultation

1995-1997	Scientific advisor at clinical trials for Parkinson's and Huntington's disease at HMS/Brigham and Women's Hospital, Boston, MA and Lahey Clinic, Burlington, MA

b) Public Presentations as an Expert in Discipline

1990	Huntington Disease Society of America, basic research presentation for patients
1994	Belmont Rotary Chapter, science presentation
1994	American Parkinson Disease Association, Local chapter for patients, Presentation on basic research towards new treatments for PD, Boston
1995	National Youth Leadership Forum, presentation to high school students interested in pursuing research and medical studies, Simmons College, Boston
1995	Advisory presentation before the US Senate Special Committee on Aging, Capitol Hill, Washington, D.C.
1995	Advisory presentation before the US House Commerce Committee, Health and Environment Subcommittee, Capitol Hill, Washington, D.C.
1996	Presentation on current PD research to patient group, Boston, MA
1996	Research Update, Parkinson's Action Network Third Annual Public Policy Forum, Washington, D.C.
1997	Advisor to members of Labor, Health and Education Committee on the progress of basic neuroscience research, Washington, D.C.
1999	Advisory presentation on neurological research to science advisors to the President at the White House, Washington, D.C.

Other Professionally Related Service

1996	National Parkinson Foundation "On the Causes and Treatments of Parkinson's Disease"
1997	ABC "Turning Point", Television interview on Neural Xenotransplantation
1997	NPR "Science Friday", Radio interview on Risks and Benefits of Xenotransplantation
1998	CBS "60 Minutes", Television interview on xenotransplantation.

EDITORIAL BOARDS:

Member of Editorial Board

1989-	Journal of Neural Transplantation and Plasticity
1991-	Cell Transplantation
1997-	Restorative Neurology and Neuroscience
1997-	Experimental Neurology
1998-	inSight

Guest Editorships

1995	Cell Transplantation, ASNT Special Issue
1996	Cell Transplantation, ASNT Special Issue
1999	Brain Pathology, Special Issue
1999	Experimental Neurology, Special Issue
2000	Neuroscience News, Special Issue

Current Editorial Review Assignments

Science, Nature, Nature Medicine, Nature Genetics, Journal of Neuroscience, Journal of Comparative Neurology, Neuroscience, Brain Research, Proceedings of the National Academy of Science USA

PART II:

REPORT OF RESEARCH:

Major Research Interests:

(1) Methods for neuronal repair, regeneration and protection: using animal models of Parkinson's, Huntington's and Alzheimer's diseases.

(2) The pathophysiology and therapy of CNS degeneration in Huntington's, Parkinson's, and Alzheimer's diseases.

Narrative Description of Research:

The research programs focus on mechanisms of neuronal degeneration and innovative methods for cellular repair, with emphasis on the neurodegenerative disorders like Parkinson's (PD), Alzheimer's (AD), and Huntington's (HD). This work has already led to applications of cell therapy for PD and HD. This laboratory is involved in several pharmacological, gene, molecular and cellular studies to obtain neuroprotection or cell and gene delivery in the CNS.

1. Neural Transplantation:

The understanding of regeneration and plasticity of the mammalian nervous system has developed over the last decade. We have participated in research that shows that the brain is a regenerative system which can integrate implanted primary neurons, progenitor or stem cells into the adult brain. These implanted neurons grow physiologically and functionally to repair previously damaged or degenerated neuronal pathways. The specific experiments performed in this laboratory have significantly influenced neural transplantation in clinical trials using porcine and human cells for Parkinson's and Huntington's disease. Encouraging results suggest that cell therapy may become, when fully developed, a useful treatment for neurodegenerative disease. At the very fundamental level, this laboratory also investigates the specific axon guidance factors that persist in the adult brain. In animal models, by transplanting neuroblasts into various locations in the brain, we are determining if reconnection and tropic interactions are possible. The goal of this work is a better understanding of the structural and functional plasticity of the central nervous system that can lead to improved therapies for neurological disease.

REPORT OF RESEARCH: (continued)

2. Neuroprotection:

Starting in 1989, this laboratory has investigated a number of paradigms in which neurotrophic factors can prevent degenerative events. Based on the theory that the neuron at any given moment is in a defined state of vulnerability (dependent on genetic and phenotypic characteristics), we have devised methods to improve the resilience of neurons affected in Huntington's, Parkinson's and Alzheimer's diseases. We are currently investigating several novel molecular (e.g. neurophilins) treatments for Parkinson's disease and Alzheimer's disease models. In addition, we are performing basic research on molecular modifications of striatal neurons to enhance their capacity to withstand insults or modify their genetically induced vulnerability in Huntington's disease.

3. Gene Delivery and Gene Therapy:

Since 1989, this laboratory has developed a number of model systems for gene delivery, ranging from delivery in the peripheral nervous system to specific delivery in anatomical systems involved in Parkinson's and Huntington's disease. We have used modified and transfected cell lines to establish new paradigms for neuroprotection and recently have made inroads into efficient, selective and stable delivery and expression of genes by modified viral vectors into the CNS.

(Please refer to Bibliography to see the development and progress of these research paths.)

Specific Research Funding Information:

1989-90	NATO Grant# CRG 890583, (PI) "Neural transplantation in primate models of neurodegenerative disease".
1990-91	NIH Program Project Award: Huntington's Disease Center, (PI of sub-contract) MGH and McLean Hospital.
1991-96	NIH: Neurological Science. Research Grant NS29178, (PI), "Excitotoxic Cortex Lesions-Degeneration and Remodeling"
1992-93	Biotechnology sponsored research program, (PI) "Cell Based Therapy"
1992-95	NIH: Neurological Science. Research Grant # RO1 NS30064, "Neuronal Replacement in a Model of Huntington's Disease
1993-94	Biotechnology sponsored research program, (PI) "Cell Based Therapy"
1994-	NIH: MH19905 (Benes, PI) "Clinical Neuroscience Training Program Grant"
1994-95	Biotechnology sponsored research program, (PI) "Immunological masking techniques in intracerebral fetal nerve cell transplantation"
1995	Milton Fund, Harvard University, (PI) "Novel neurotrophic molecules for neuroprotection in Parkinson's disease"
1995-96	Wills Foundation, (Sponsor (OI), Haque, fellowship),
1995-96	Biotechnology sponsored research program, (PI) "New Immunological Methods in Intracerebral Fetal Nerve Cell Transplantation"
1995-98	NIH: Neurological Science. Research Grant # RO1 NS30064, Competing Renewal, (PI) "Neuronal Replacement in a Model of Huntington's Disease"
1996-	NIH: 5-T32-AG00222 (Yankner, PI) "Molecular Biology of Neurodegeneration Training Grant"
1996-97	National Parkinson Foundation, (Sponsor (OI), Costantini, fellowship) "Ex vivo and in vivo gene transfer of CuZn SOD-1: protection from oxidative stress and neurodegeneration"
1996-97	Biotechnology sponsored research program, (PI) "Cholinergic neuronal replacement in animal models"
1996-	Pharmaceutical industry sponsored research program, (PI) "Documentation of neuroprotective and therapeutic effects of compounds against MPTP/MPP+ induced neurotoxicity in mice and rat paradigms of Parkinson's disease"
1997-	Biotechnology sponsored research program, (PI) "Functional pharmacological and therapeutic models relevant to neuroprotection or amyloid formation"
1997-	Biotechnology sponsored research program, (PI) "Identification of the human immune response to porcine fetal mesencephalic cells"

Research Funding Information: (continued)

1997-99	Clinical Neuroscience Training Program, (Sponsor (OI), Costantini, fellowship) "Ex vivo and in vivo gene transfer of CuZn SOD-1: protection from oxidative stress and neurodegeneration"
1998-	Federal, USAMRAA, DAMD17-98-1-8618, (PI) "Structural and functional brain repair studies of PD models by novel neurosurgical, PET and MRI/MRS methods"
1999-	Federal, USAMRAA, DAMD17-99-1-9482, New (PI) "Knock-out and transgenic strategies to improve neural transplantation therapy for Parkinson's disease"
1999-	Century Foundation, Sarasota Memorial Hospital, sponsored research program (PI) "Electrophysiology of PD"
1999-	Federal: NINDS/NIH P50 NS39793, Parkinson's disease Research Center of Excellence (Center Director and PI) "Novel Therapeutic Approaches to Parkinson's disease"
1999-	NIH: Neurological Science. Research Grant # RO1 NS30064, Competing Renewal, (PI) "Transgenic Xenotransplants for Huntington's Disease"
2000-	NIH Neurological Science. Research Grant # RO1 NS41263-01, "Novel Anti-inflammatory Therapies for Neurotoxically Induced PD"
(2000)	*Total Grant Support: $2,200,000/yr.*

REPORT OF TEACHING:

Local Contributions:

Graduate Medical Courses/Seminars/Invited Teaching Presentations:

1988	Boston, Dept. of Neurology, Harvard Medical School, Massachusetts General Hospital "Neuronal transplantation and strategies for CNS regeneration" (seminar)
1990-	Faculty, Program of Neuroscience, Harvard Medical School. Faculty, Neurobiology of Behavior course/program, McLean Hospital, Harvard Medical School
1990	Boston, MA "Excitotoxic lesions of the cerebral cortex model degeneration and plasticity seen in neurodegenerative diseases" (lecture)
1990	Cold Spring Harbor, N.Y., "The use of genetically engineered cells as donor tissue in models of intracerebral transplantation" (lecture)
1990	Woods Hole Marine Biology Laboratory, RUNN course lecture: "Studies of neuronal cell death and regeneration in transplantation models" (faculty)
1992	Course organizer: HMS Program of Neuroscience Course; "Paradigms to investigate neuronal health: what happens to neurons in neurodegenerative diseases"
1992	Faculty, Lecturer, Dept. of Neurology, HMS, MGH course: "Intensive Clinical and Basic Neuroscience Update"
1993	Neurobiology 209, Harvard Medical School (lecture)
1994	Massachusetts General Hospital, Scientific Integrity Course (faculty)
1995	McLean Hospital, Clinical Neuroscience Training Program,"Neural transplantation: site-specific CNS delivery of neuroprotectants and neurotransmitters" (lecture)
1996	Gene Therapy: Principles and Practice (Genetics 208), Harvard Medical School "Strategies of gene therapy for dominant and recessive genetic, as well as non-hereditary, diseases" (lecture)
1996	Neurobiology of Disease Course, Harvard Medical School
1997	McLean Hospital, Clinical Neuroscience Training Program, "Neural transplantation: site-specific CNS delivery of neuroprotectants and neurotransmitters" (lecture)
1997	Boston, Brigham and Women's Hospital, "Hosting foreign cells in the brain: will xenogeneic neurons serve as treatments for Parkinson's and Huntington's disease?" (lecture)
1997	Gene Therapy Group, Harvard Medical School "Gene Therapy for Huntington's Disease" (lecture)
1997	Boston, Harvard-Mahoney Neuroscience Institute Forum, "Neural Transplantation in Parkinson's and Huntington's Disease"

TEACHING: Local Contributions: (continued)

Graduate Medical Courses/Seminars/Invited Teaching Presentations: (continued)

1998	Boston, MGH, Current Approaches to Understanding Neurodegenerative Disease Symposium, "Neural transplantation therapy for neurodegenerative disease". (lecture)
1999	Southborough, MA, Harvard Primate Research Center, "How neural transplantation can work in patients with neurodegenerative disease" (lecture)
1999	Boston, Partners Neurology Residency, Basic Neuroscience Course, "Neuronal Death, Regeneration and Transplantation" (lecture)
1999	McLean Hospital, Clinical Neuroscience Training Program, "Neural transplantation: site-specific CNS delivery of neuroprotectants and neurotransmitters" (lecture)
2000	Cambridge, MIT, Modulation of APP and memory by the cholinergic system (lecture)

Continuing Medical Education Courses:

1992	Dept. of Neurology, HMS, MGH course, Boston,MA "Intensive Clinical and Basic Neuroscience Update" (faculty)
1996	State University of New York, Syracuse, NY, 8th Annual Neuroscience Symposium (Neurofest '96), "Specificity of axonal growth from porcine embryonic neural xenografts in host brain" (lecture)
1998	Co-director, "Anatomy and Physiology of Basal Ganglia Surgery" CME Course and Scientific Conference, Sarasota, FL
1998	Organizer, Second "Cellular and Molecular Treatments of Neurological Diseases" Scientific Conference, Cambridge, MA
1999	Co-director, "Anatomy and Physiology of Basal Ganglia Surgery" CME Course and Scientific Conference, Sarasota, FL

Advisory and Supervisory Responsibilities in Clinical or Laboratory Setting:

A. Faculty Mentor, Thesis Advisor or Supervisor in Neuroregeneration Laboratories:

A1. Current Predoctoral Students: (Degree)

2000-Present	Therese Andersson	MSc. 2000 Kalmar University
1999-Present	Anna Moore	MSc. 1998 Cardiff University

A2. Past Predoctoral Students: (Degree, current position)

1997-1999	Anna Mattsson	MSc. 1998 Kalmar University, PhD Student, Karolinska Institute
1997-1999	Biljana Georgievska	MSc. 1998 Kalmar University, PhD Student, Lund University
1997-1998	Karin Holm	MD 1999 Lund University, Resident, Lund University
1997-1998	Zita Boonman	MD 1999 Utrecht University, Resident, Utrecht University
1996-1997	Lina Fine	BS 1997 Harvard University, PhD Studies
1996-1997	Brandi Whatley	BS 1997 Boston University, Ph.D. Student, U. Maryland
1993-1996	Wendy Galpern	BS 1989 Tufts University, PhD 1996, NRL/McLean Hospital & U Mass, MD 1998 U Mass Resident Neurology MGH Instructor, Harvard Medical School
1995-1996	Arif Husain	BS 1996 MIT, Medical School
1995-1996	Paul Borghesani	BS 1994 MIT, MD-PhD 1999, Harvard Medical School
1995	Marcus Ware	BS 1993 Tougaloo College, MD-PhD at Harvard/MIT HST Program

TEACHING: Local Contributions: (continued)

A2. Past Predoctoral Students: (Degree, current position) (continued)

1995	Amy Spiegel	BS 1995 West Chester University, Medical School
1995-1996	D. Andrina Ngo	BS 1996 Harvard University, PhD Student, Johns Hopkins University
1994	Lisa Genova	Program in Neuroscience, PhD 1998 Harvard Medical School, Consultant, Health Advances, Wellsley
1993-1995	David St. Peter	BS 1995 Harvard University, Director, Biotech. development, New York, NY
1993-1995	Antony Garcia	BS 1995 Harvard University, Director, Computer-software development, Cambridge, MA
1993-1994	Marc Dinkin	BS 1994 Harvard University, Medical School
1992-1993	Tara Uhler	MA 1992 Harvard University, MD, Harvard Medical School
1991-1992	Joseph Simpson	BS 1992 Harvard University, MD, PhD at Washington University, St. Louis
1991-1992	Wendy Yee	BS 1992 MIT, 1999, PhD at Johns Hopkins University
1989-1991	Lisi Fishman	BS 1991 Harvard University, Psychologist, Cambridge, MA

A3. Current Staff and Postdoctoral Fellows:

2000-	Kai Sonntag	MD 1993, PhD 1994 U. Heidelberg, Instructor HMS
2000-	Aygul Balcioglu	PhD 1994 Mass College of Pharmacy, Instructor HMS
2000-	Kevin McNaught	PhD 1995 Kings College, London
2000-	Hyemyung Seo	PhD 1999 U. Tennessee, College of Medicine
2000-	Lars Bjorklund	MD 1999, PhD 1998 Karolinska Institute, Sweden
1999-	Sangmi Chung	PhD 1998 Cornell University, New York
1999-	Francesca Cicchetti	PhD 1998 Robert Laval Univ., Quebec
1998-	Anna-Liisa Brownell	PhD 1974 University of Helsinki Asst. Imaging Biologist, McLean Hosp. Assoc. Prof., Harvard Medical School
1998-	Craig van Horne	MD 1992 U. Colorado School of Medicine PhD 1992 U. Colorado School of Medicine Assistant Neurobiologist, McLean Hospital Asst. Prof., Harvard Medical School
1996-	Wendy Galpern	Ph.D. 1996 U Mass/McLean/NRL Instructor, 1997 Harvard Medical School

A4. Past Postdoctoral Fellows: (Degree, current position)

1996-2000	Lauren Costantini	Ph.D. 1996 U. of New York, Albany Assoc. Director of Technol. Dev., Titan, Inc.
1996-1999	Ling Lin	M.D. 1985 Henan Medical U. Graduate School, Hong Kong
1992-1999	Terry Deacon	Ph.D. 1984 Harvard U., Associate Professor Boston University (formerly Assoc. Prof. at Harvard U.)
1998-1999	Pushpa Tandon	Ph.D. 1985 U. Lucknow, India Research Scientist, Igen, Inc.
1995-1996	Nadia S. K. Haque	Ph.D. 1995 Cambridge U., Research Scientist, Geron, Inc.
1992-1996	Lindsay Burns	Ph.D. 1991 Cambridge U., Research Scientist, Neurex, Inc.

TEACHING: Local Contributions: (continued)

A4. Past Postdoctoral Fellows: (Degree, current position)(continued)

1993-1995	Stephen Tatter	M.D./Ph.D. 1990, 1989 Rockefeller U. /Cornell Med. School, Asst. Prof. Neurosurgery, North Carolina Baptist Hospital
1992-1994	Peyman Pakzaban	M.D. 1984 MGH, Neurosurgery, U. Texas Med. Center
1991-1993	David Frim	Ph.D./M.D. 1988 Harvard Medical School , Neurosurgery, Assoc. Prof., U. Chicago Medical Center
1991-1992	Ullrich Wullner	M.D.-Ph.D. 1989 U. Gottingen, Asst. Prof., Lab Director, Neurology, University of Tübingen
1991-1992	Philippe Hantraye	Ph.D. 1987 U. of Paris Assoc. Prof., Director, Research Unit, Centre National de Recherche Scientifique (France)
1990-1992	William Rosenberg	M.D. 1987 Harvard Medical School, Asst. Prof., Neurosurgery, U. Cincinnati
1989-1990	James Schumacher	M.D. 1986 U. Washington, Neurosurgery, Sarasota Memorial Hospital Instructor, Harvard Medical School 2000

B. Recent Theses/Dissertations Directed:

B1. Graduate

Anna Mattsson, MSc. Thesis, 1998
Biljana Gjorgijevska, MSc. Thesis, 1998
Karin Holm, MSc., MB Thesis, 1998
Wendy R. Galpern, Ph.D. "Neuroprotection and Neurotransplantation Strategies in Models of Parkinson's Disease", Awarded 1996

B2. Undergraduate (Primary research supervisor and mentor roles)

Brandi Whatley, BS, Senior Honors Thesis, 1997 Boston University
Amy Spiegel, BS, Senior Honors Thesis, 1995 West Chester University
David St. Peter, BS, Senior Honors Thesis, Biology, 1995 Harvard University
Antony Garcia, BS, Senior Honors Thesis, Biology, 1995 Harvard University
Marc Dinkin, BS, Senior Honors Thesis, Biology, 1994 Harvard University
Joseph Simpson, BS, Senior Honors Thesis, Biology, 1992 Harvard University
Wendy Yee, BS, Senior Honors Thesis, Biology, 1992 MIT
Lisi Fishman, BS, Senior Honors Thesis, Biology, 1991 Harvard University

C. PhD Dissertation or Examining Committees:

1993-1995	Macrene Alexiades, Thesis Advisory Committee, HMS
1994	Wendy R. Galpern, Oral Qualifying Examination Committee, U Mass Medical School
1994-1996	Paul Borghesani, Thesis Advisor, MD-PhD Program, HMS
1999	Richard Christie, PhD Thesis Examination Committee, HMS

Leadership Roles:

1992-1993	"On Neuronal Health", Graduate course, HMS (organizer)
1994	HMS Neuroscience Student Visit to McLean Hospital (organizer)
1995	National Youth Leadership Forum, presentation to high school students interested in pursuing research and medical studies, Simmons College, Boston
1995	Scientific Program Organizer, "Cellular and Molecular Treatments of Neurological Diseases" Conference, Three day international conference of 50 invited participants including students from HMS Program in Neuroscience and residents of Neurology and Neurosurgery Programs at HMS hospitals.

TEACHING: Local Contributions: (continued)

Leadership Roles: (continued)

1998	Scientific Program Organizer, "Cellular and Molecular Treatments of Neurological Diseases" Conference, American Academy of Arts and Sciences, Cambridge, MA. Three day international conference of 150 invited participants including students from HMS Program in Neuroscience and residents of Neurology and Neurosurgery Programs at HMS hospitals

TEACHING: National or International Contributions:

Medical/Graduate School Courses/Seminars/Invited Teaching Presentations:

1981-83	Seminars and tutorials in Cell Biology, Histology and Neurobiology at the Medical Faculty, University of Lund, Sweden
1983-85	Lecturer in Neurobiology and Histology at the Medical Faculty, University of Lund.
1985-87	Lecturer and Assistant Director of Medical Neurobiology Course, Lecturer in Histology. Supervisor for research students in Medicine, Co-supervisor for 2 PhD students, University of Lund.
1987-89	V. Fellow, Jesus College, Cambridge, University of Cambridge, England. Supervisor for Medical Part II students, University of Cambridge, England.
1990	Faculty, Lecturer, RUNN Course (Review and Update in Neurobiology for Neurosurgeons and Neurologists) Woods Hole, MA
1990	Faculty, Lecturer, Cold Spring Harbor Course: "Molecular Genetic Analysis of Diseases of the Nervous System", N.Y.
1994	Woods Hole, MA, RUNN Course "Affecting Neural Function by Transplantation" (faculty)
1995	Chicago, IL, for Rush University Research Week (Keynote speaker)
1996	Miami, FL, The University of Miami, The Miami Project to Cure Paralysis "Specificity of connections formed by transplanted fetal neurons to the mature CNS" (Visiting Professor)
1998	Karolinska Institute, Stockholm, Introductory lecture for PhD thesis examination for Lars Bjorklund on Intraocular Cellular Transplants

Invited Presentations:

1983	Hamburg, European Neuroscience Association "Monitoring of neuronal survival in suspensions of embryonic CNS tissue" (paper)
1984	University of Cambridge, Downing Site "Functional neuronal replacement in the ibotenic acid lesioned neostriatum by neostriatal neural grafts" (lecture)
1984	Lund, Nordic Meeting in Neuropsychiatry "Functional neuronal replacement in an animal model of Huntington's disease" (paper)
1984	Oxford, Dept. of Pharmacology, University of Oxford "Striatal neural transplant in the excitotoxically lesioned neostriatum" (lecture)
1985	Uppsala, Nordic Physiology Meeting "Neuronal replacement in an animal model of Huntington's disease (paper)
1985	Munchen, Glial-neuronal communication symposia "The use of neural transplants in the study of lesion models of the adult CNS" (lecture)
1985	Oxford, European Neuroscience Association "Morphological and behavioural changes following neural grafting in rats with lesions of the anteromedial neostriatum" (paper)
1986	Avoriaz, Symposium at European Winter Congress on Brain Research "Neural replacement by intracerebral grafts in animal models of Parkinson's and Huntington's disease" (chairman and lecture)
1986	New York, New York Academy of Sciences "Morphology and function of striatal neural grafts" (lecture)
1986	Dusseldorf, Dept. of Neurology "The use of neural grafting in studies of CNS development and regeneration" (lecture)

TEACHING: National or International Contributions: (continued)

Invited Presentations: (continued)

1986	Spetses-ETP, Research program at European Training Program "Autumn School" "The use of neural grafting in experimental studies of CNS regeneration and development" (lecturer)
1987	London, Royal Free Hospital, Dept. of Psychiatry "Aspects of degeneration and regeneration in the adult CNS using intracerebral transplants" (lecture)
1987	London, Maudsley Hospital, Inst. of Psychiatry "Neural grafting in animal models of neurodegenerative disease" (lecture)
1987	Venice, 2nd Symposium on Restorative Neurology "The use of fetal neurons to replace neurons in the CNS" (lecture)
1987	Rochester, New York, at Neural transplantation into the mammalian CNS meeting, "Fetal cortical grafts into the excitotoxically lesioned neocortex: a model for trophic interactions in Alzheimer's disease ?" (paper)
1987	Pécs, Hungary, Satelite Symposium on Neural Regeneration and Transplantation "Striatal cell suspension grafts in an animal model of Huntington's disease" (paper)
1987	Paris, Dept of Neurology, Fréderic Joliot Hospital, Orsay " A primate model of Huntington's disease" (lecture)
1988	Paris, Dept. of Neurology, Frederic Joliot Hospital, Orsay "Excitotoxic lesions models of CNS degeneration" (lecture)
1988	Paris, Dept. of Neurology, Frederic Joliot Hospital, Orsay "The use of neural transplantation in patients with neurodegenerative disease: basic research and recent clinical experiments" (lecture)
1988	Lyon, Conference: Trends in Neurobiology "Neuron-target interaction in the CNS: neuronal degeneration and regeneration theories" (paper)
1989	Cambridge, England, Neural transplantation meeting: molecular bases to clinical application "Neural transplantation in a primate model of Huntington's disease" (paper)
1990	Lund, Sweden "From pharmacological to neuronal replacement in Huntington's disease" (paper)
1991	St. Louis, Missouri, CNS Transplants in Adult Damaged Sensory Thalamus and Neocortex (lecture)
1991	Washington, D.C., Georgetown University, Neural Transplantation in Animal Models of Huntington's Disease (lecture)
1991	Paris, La Salpetriere Hospital, "Animal Models of Neuronal Protection, Degeneration and Regeneration: Concepts of Neuronal Health" (lecture)
1991	Stockholm, Karolinska Institute, "CNS degeneration and regeneration models: new concepts of neuronal damage and protection" (lecture)
1992	Nagoya, Japan, "International Conference on Biochemistry of Disease" (lecture)
1992	Washington, D.C. "IV International Symposium on Neural Transplantation" (lecture)
1992	Brussels, "25th International Congress of Psychology" (lecture)
1993	Frankfurt, Symposium on anti-excitotoxic therapy: "Neuronal protection, gene-transfer and circuitry repair in the basal ganglia" (lecture)
1994	Hancock, MA, Third Berkshire Neuroscience Symposium (lecture)
1994	Chatenay-Malabry (Paris), 5th International Symposium on Neural Transplantation (lecture)
1995	Winter Conference on Brain Research, "Primate models of caudate-putamen motor functions" (lecture)
1995	Paris, ANPP Meeting "Novel Therapeutics in the Nervous System: Gene Transfers and Trophic Factors" (lecture)
1995	Philadelphia, PA, Intl. Conf. on Gene Therapy for CNS Disorders, "Gene Therapy for Huntington's Disease" (lecture)
1995	National Press Club, Washington D.C. "New therapies for Parkinson's disease" (lecture)
1995	U.S. Senate Special Committee on Aging, Washington D.C. Advisory presentation on Parkinson's disease
1995	House Subcommittee on Health and Environment, Washington, D.C., Advisory presentation on Parkinsons disease
1995	Maastricht, Holland, Annual Meeting of NECTAR (lecture)

TEACHING: National or International Contributions: (continued)

Invited Presentations: (continued)

1996	Washington, DC, National Foundation for Brain Research, Gene Therapy for Parkinson's Disease Consortium
1996	San Francisco, CA, Annual Meeting of American Diabetes Association "Neural xenotransplants in degenerative disease" (lecture)
1996	Miami, FL, The University of Miami, The Miami Project to Cure Paralysis "Specificity of connections formed by transplanted fetal neurons to the mature CNS" (Visiting Professor)
1996	New York, NY, New York Academy of Sciences "Cellular protection and repair of the brain using cell transplantation" (lecture)
1996	New Haven, CT, Yale University, Ninth Gene Therapy User Group Meeting, "Gene delivery strategies for the treatment of neurodegenerative diseases" (lecture)
1997	Denver, CO, Univ. of Colorado "Hosting foreign cells in the brain: will xenogeneic neurons serve as treatments for Parkinson's and Huntington's disease?" (lecture)
1997	U.S. Veterans Administration, Washington, D.C. (1997) Chairman, Advisory Committee on Parkinson's disease research
1998	Austrian Parkinson Society, Vienna, "Reconnections of neural circuitry in Parkinson's disease patients by xenogeneic dopaminergic neurons." (lecture)
1998	Karolinska Institute, Stockholm, Introductory lecture for thesis examination on Intraocular Cellular Transplants
1998	New York, NY, 5th Intl. Congress of Parkinson's Disease and Movement Disorders. "Gene Therapy for Parkinsons' Disease", (plenary lecture)
1998	Tokyo, Japan,The Molecular Medicine Revolution Conference, "Neural cell transplants to physiologically repair circuitry in neurodegenerative diesease" (plenary lecture)
1998	Cardiff, Wales, The Physiological Society, "Cell transplantation as a therapy for Parkinson's disease" (lecture)
1999	Cornell Medical School/New York Hospital "Developing nerve cells against neurodegeneration" (grand rounds & lecture)
1999	Montreux, Switzerland, The International Cell Transplant Society, "Primary neuronal cell transplantation for Parkinson's disease (lecture)
1999	Keystone Symposia, "Neural xenotransplantation for neurodegenerative disease" (lecture)
1999	Dalhousie University, Halifax, Clinical Neuroscience (rounds) and Dept. of Anatomy and Neurobiology (lecture)
1999	University of Pittsburgh Medical Center, Dept. of Pathology (lecture)
1999	University of Rochester, Experimental Therapeutics Workshop (lecture) and Neurology Grand Rounds
1999	Vancouver, BC, XIIIth Intl. Congress on Parkinson's Disease (lecture)
1999	Odense, Denmark, 7th Intl. Neural Transplantation Meeting (lecture)
1999	Boston, European Behavioral Pharmacology Society and Behavioral Pharmacology Society Conference (lecture)
1999	Austrian Parkinson Society, Vienna (lecture)
1999	Bonn, Intl. Neuroscience Symposium "Molecular Basis of CNS Disorders" (lecture)
1999	London, The Novartis Foundation "Neural Transplantation in Neurodegenerative Disease"
1999	Miami, 6th National Parkinson's Foundation Intl. Symposium on Parkinson's Research (lecture)
2000	Louisville, "The Neuroscience of Developing Cell Therapies for Parkinson's Disease" (lecture)
2000	Zurich, Intl. Study Group on the Pharmacology of Memory, (lecture)
2000	Tokyo, Intl. Workshop: Stem Cell Biology & Cellular Molecular Treatment (lecture)
2000	Il Ciocco, Italy, Gordon Research Conference (lecture)
2000	Rome, Intl. Cong. of the Transplantation Society (lecture)
2000	Turin, Italy, Cellular & Molecular Mechanisms of Brain Repair (lecture)

TEACHING: National or International Contributions: (continued)

Advisory and Supervisory Responsibilities in Clinical or Laboratory Setting:

A. Faculty Mentor, Thesis Advisor or Supervisor:

1988-1989	Boris Lambs	MD 1990 Cambridge U. MD at Cambridge University, England
1986-1988	Walter Fischer	MD 1991 Lund University MD, PhD at Lund University Medical School, Sweden
1986-1988	Klas Wictorin	MD 1991 Lund University MD, PhD at Lund University Medical School, Sweden
1985-1986	Lars Anderson	MD 1989 Lund University MD at Lund University Medical School, Sweden

B. Theses/Dissertations Directed:
Walter Fischer, PhD at Lund University, (Asst. Supervisor) Awarded 1988
Klas Wictorin, PhD at Lund University, (Asst. Supervisor) Awarded 1988
Boris Lambs, MA at Cambridge U., (Co-advisor) Awarded 1989

C. Ph.D. Dissertation or Examining Committees:

1994	Principal examiner, Serge Marty's Doctoral Thesis, INSERM, Paris, France
1998	Principal examiner, Lars Björklund's Doctoral Thesis, Karolinska Institute, Sweden

Professional Leadership Roles related to Teaching:

1995-1996	Chair, Program Committee, American Society for Neural Transplantation
1995	Organizer, "Cellular and Molecular Treatments of Neurological Diseases" Conference, Three day international conference with invited speakers and participants, Cambridge, MA, (Sept. 7-10, 1995)
1998	Co-director, "Anatomy and Physiology of Basal Ganglia Surgery" CME Course and Scientific Conference, Sarasota, FL (March 13-15 1998)
1998	Organizer, "2nd Cellular and Molecular Treatments of Neurological Diseases" CME Course and Scientific Conference, Cambridge, MA (Oct. 8-11, 1998)
1999	Co-director, "2nd Anatomy and Physiology of Basal Ganglia Surgery" CME Course and Scientific Conference, Sarasota, FL (May 15-16, 1999)

CLINICAL ACTIVITIES: (N/A)

(Dr. Isacson does not have a clinical appointment or license.)

1995-1997	Scientific advisor at clinical trials for Parkinson's and Huntington's disease at HMS and Lahey Clinic, Burlington, MA

PART III: BIBLIOGRAPHY (172 Total: 104 Original, 66 Reviews/Chapters, 2 Books)

Original Reports

1. Isacson O, Brundin P, Kelly PAT, Gage, FH and Björklund A. Functional neuronal replacement by grafted striatal neurons in the ibotenic acid lesioned rat striatum. Nature 1984;311:458-60.

2. Gage FH, Dunnett SB, Brundin P, Isacson O, Björklund A. Intracerebral grafting of embryonic neural cells into the adult host brain: an overview of the cell suspension method and its application. J Dev Neurosci 1984;6:137-51.

3. Brundin P, Isacson, O, Björklund A. Monitoring of cell viability in suspensions of embryonic CNS tissue and its use as a criterion for intracerebral graft survival. Brain Res 1985;331:251-9.

4. Isacson O, Brundin P, Gage FH, Björklund A. Neural grafting in a rat model of Huntington's disease: Progressive neurochemical changes after neostriatal ibotenate lesions and striatal tissue grafting. Neuroscience 1985;16:799-817.

5. Gage FH, Brundin P, Isacson O, Björklund A. Rat fetal brain tissue survive and innervate host brain following five day pregraft tissue storage. Neuroscience Lett 1985;60:133-7.

6. Brundin P, Barbin G, Isacson O, Mallat M, Chamak B, Prochiantz A, Gage FH Björklund A. Survival of intracerebrally grafted rat dopamine neurons previously cultured in vitro. Neuroscience Lett 1985;61:79-84.

7. Zetterström T, Brundin P, Gage FH, Sharp T, Isacson O, Dunnett SB, Ungerstedt U, Björklund A. In vivo measurement of spontaneous release and metabolism of dopamine from intrastriatal nigral grafts using intracerebral dialysis. Brain Res 1986;362:344-9.

8. Isacson O, Dunnett SB, Björklund A. Behavioural recovery in an animal model of Huntington's disease. Proc Natl Acad Sci USA 1986;83:2728-32.

9. Brundin P, Isacson O, Gage FH, Björklund A. Intrastriatal grafting of dopamine-containing neuronal cell suspensions: effects of mixing with target or non-target cells. Dev Brain Res 1986;24:77-84.

10. Brundin P, Isacson O, Gage FH, Prochiantz A, BjörklundA. The rotating 6-hydroxydopamine lesioned mouse as a model for assessing functional effects of neuronal grafting. Brain Res 1986;366:346-49.

11. Sofroniew MV, Isacson O, Björklund A. Cortical grafts prevent atrophy of cholinergic basal nucleus neurons induced by excitotoxic cortical damage. Brain Res 1986;378:409-15.

12. Sofroniew MV, Pearson RCA, Isacson O, Björklund A. Experimental studies on the induction and prevention of retrograde degeneration of basal forebrain cholinergic neuron. Prog Brain Res 1986;70: 363-89.

13. Pritzel M, Isacson O, Brundin P, Wiklund L, Björklund A. Afferent and efferent connections of striatal grafts implanted into the ibotenic acid lesioned neostriatum in adults rats Exp Brain Res 1986;65:112-26.

14. Dunnett SB, Whishaw IQ, Jones GH, Isacson O. Effects of dopamine-rich grafts on conditioned rotation in rats with unilateral 6-hydroxydopamine lesions. Neurosci Lett 1986;68:127-33.

15. Isacson O, Dawbarn D, Brundin P, Gage FH, Emson PC, Björklund A. Neural grafting in a rat model of Huntington's disease: Striosomal organization as revealed by immunocytochemistry, acetylcholinesterase histochemistry, and receptor autoradiography. Neuroscience 1987;22:481-97.

16. Isacson O, Fischer W, Wictorin K, Dawbarn D, Björklund A. Astroglial response in the excitotoxically lesioned neostriatum and its projection areas. Neuroscience 1987;20:1043-56.

PART III: BIBLIOGRAPHY (172 Total: 104 Original, 66 Reviews/Chapters, 2 Books)

Original Reports (continued)

17. Peschanski M, Isacson O, Fetal homotypic transplants in the excitotoxically neuron depleted thalamus I: Light microscopy. J Comp Neurol 1988;274:449-63.

18. Clarke DJ, Dunnett SB, Isacson O, Sirinathsinghji DJS, Björklund A. Striatal grafts in rats with unilateral striatal lesions I:Ultrastructural evidence of afferent synaptic inputs from the host nigrostriatal pathway. Neuroscience 1988;24:791-801.

19. Sirinathsinghji DJS, Dunnett SB, Isacson O, Clarke DJ, Björklund A. Striatal grafts in rats with unilateral neostriatal lesions II: In vivo monitoring of GABA release in the globus pallidus and substantia nigra. Neuroscience 1988;24:803-11.

20. Dunnett SB, Isacson O, Clarke DJ, Björklund A. Striatal grafts in rats with unilateral striatal lesions III: recovery from dopamine dependent motor asymmetry and deficits in skilled paw reaching. Neuroscience 1988;24:813-20.

21. Brundin P, Barbin G, Strecker RE, Isacson O, Prochiantz A, Björklund A. Survival and function of dissociated rat dopamine neurones grafted at different developmental stages or after being cultured in vitro. Dev Brain Res 1988;39 233-43.

22. Peschanski M, Rudin M, Isacson O, Delepierre M, Roques B. Magnetic resonance imaging of intracerebral neural grafts. Prog Brain Res 1988;78:619-25.

23. Isacson O, Wictorin K, Fischer W, Sofroniew M, Björklund A. Fetal cortical suspension grafts to the excitotoxically lesioned neocortex: anatomical and neurochemical studies of trophic interactions. Prog Brain Res 1988;78:13-27.

24. Fischer W, Wictorin K, Isacson O, Björklund A. Trophic effects on cholinergic striatal interneurones by submaxillary gland transplants. Prog Brain Res 1988;78:409-13.

25. Wictorin K, Isacson O, Fischer W, Nothias F, Peschanski M, Bjorklund A. Connectivity of striatal gratfts implanted into the ibotenic acid-lesioned striatum I: subcortical afferents. Neuroscience 1988;27:547-62.

26. Nothias F, Wictorin K, Isacson O, Björklund A, Peschanski M. Morpological alteration of thalamic afferents in the excitotoxically lesioned striatum. Brain Res 1988;461:349-54.

27. Lams BE, Isacson O, Sofroniew MV. Loss of transmitter-associated staining following axotomy does not indicate death of brainstem cholinergic neurons. Brain Res 1988;475:401-6.

28. Sofroniew MV, Isacson O. Distribution of degeneration of cholinergic neurons in the septum following axotomy in different portions of the fimbria fornix: a correlation between the degree of cell loss and the proximity of neuronal somata to the lesion. J Chem Neuroanat 1988;1:327-37.

29. Sofroniew MV, Isacson O, O'Brien TS. Nerve growth factor receptor immunoreactivity in the rat suprachiasmatic nucleus. Brain Res 1989;476: 358-62.

30. Wictorin K, Simerly RB, Isacson O, Swanson LW, Bjorklund A. Connectivity of striatal grafts implanted into ther ibotenic acid lesioned striatum II: efferent projecting graft neurons and their relationship to host afferents within the grafts. Neuroscience 1989;30:313-30.

31. Isacson O, Riche D, Hantraye P, Sofroniew MV, Maziere M. A primate model of Huntington's disease: cross-species implantation of striatal precursor cells to the excitotoxically lesioned baboon caudate-putamen. Exp Brain Res 1989;75 213-20.

PART III: BIBLIOGRAPHY (172 Total: 104 Original, 66 Reviews/Chapters, 2 Books)

Original Reports (continued)

32. Dusart I, Isacson O, Nothias F, Gumpel M, Peschanski M. Schwann cells migrate into CNS excitotoxic lesions. Neurosci Lett 1989;105:246-50.

33. O'Brien TS, Svendsen CN, Isacson O, Sofroniew M. Loss of true blue labelling from the medial septum following transection of the fimbria-fornix; evidence for the death of cholinergic and non-cholinergic neurons. Brain Res 1990;508:249-56.

34. Isacson O, Hantraye P, Maziere M, Sofroniew MV, Riche D. Apomorphine-induced dyskinesias after excitotoxic caudate-putamen lesions and the effects of neural transplantation in non-human primates Prog Brain Res 1990;82:523-33.

35. Hantraye P, Riche D, Maziere M, Isacson O. An experimental primate model for Huntington's disease: anatomical and behavioural studies of unilateral excitotoxic lesions of the caudate-putamen in the baboon. Exp Neurol 1990;108:91-104.

36. Sofroniew MV, Galletly N.P, Isacson O, Svendsen CN. Adult basal forebrain neurons do not require target neurons for survival. Science 1990;247:338-42.

37. Denys A, Leroy-Willig A, Hantraye P, Riche D, Isacson O, Maziere M, Syrota A. In Vivo MRI of neural transplants in a primate model of Huntington's disease. Amer J of Roent 1991;158 215-16.

38. Schumacher JM, Short MP, Hyman BT, Breakefield XO, Isacson O. Intracerebral Implantation of Nerve Growth Factor-Producing Fibroblasts Protects Striatum Against Neurotoxic Levels of Excitatory Amino Acids. Neuroscience 1991;45:561-70.

39. Levisohn A, Isacson O. Excitotoxic lesons of the rat entorhinal cortex. Effects of selective neuronal damage on acquisition and retention of a non-spatial reference memory task. Brain Res 1991;564:230-44.

40. Isacson O. Peschanski M. Is There Capacity for Anatomical and Functional Repair In The Adult Somatosensory Thalamus? Exp Neurol 1992;115:173-6.

41. Hantraye P, Loc'h C, Maziere B, Khalili-Varasteh M, Crouzel C, Fournier D, Yorke J-C, Stulzaft O, Riche D, Isacson O, Maziere M. 6-[18F] Fluoro-L-Dopa uptake and [76Br] bromolisuride binding in the excitotoxically lesioned caudate-putamen of nonhuman primates studied using positron emission tomography. Exp Neurol 1992;115:218-27.

42. Hantraye P, Riche D, Maziere M, Isacson O. Intrastriatal Grafting of Cross-Species Fetal Striatal Cells Reduces Abnormal Movements in a Primate Model of Huntington's Disease. Proc Natl Acad Sci USA 1992;89:4187-91.

43. Isacson O, Sofroniew MV. Neuronal loss or replacement in the injured adult cerebral neocortex induce extensive remodeling of intrinsic and afferent neural systems. Exp Neurol 1992;117:151-75.

44. Hantraye P, Brownell A-L, Elmaleh D, Spealman RD, Wullner U, Brownell GL, Madras BK, Isacson O. Dopamine fiber detection by 11C-CFT and PET in a primate model of Parkinsonism. NeuroReport 1992;3:265-8.

45. Schumacher JM, Hantraye P, Brownell A-L, Riche D, Madras BK, Davenport PD, Maziere M, Elmaleh DR, Brownell GL, Isacson O. Stereotactic CT-guided lesion method and CNS transplantation in a primate model of Huntington's disease. Cell Transplant 1992;1:313-22.

46. Rosenberg WS, Breakefield, XO, DeAntonio C, Isacson O. Detection of the E. coli lacZ gene product in the rat brain by histochemical methods. Mol Brain Res 1992;16:311-5

PART III: BIBLIOGRAPHY (172 Total: 104 Original, 66 Reviews/Chapters, 2 Books)

Original Reports (continued)

47. Beal MF, Swartz KJ, Isacson O. Developmental changes in brain kynurenic acid concentrations. Dev Brain Res 1992;68:136-9.

48. Hantraye P, Leroy-Willig A, Denys A, Riche D, Isacson O, Maziere M, Syrota A. Magnetic resonance imaging to monitor pathology of caudate-putamen after excitotoxin-induced neuronal loss in the non-human primate brain. Exp Neurol 1992;118:18-23.

49. Frim DM, Short MP, Rosenberg WS, Simpson J, Breakefield XO, Isacson O. Local protective effects of nerve growth factor-secreting fibroblasts against excitotoxic lesions in the rat striatum. J Neurosurg 1992;78:267-73.

50. Yee WM, Frim DM, Isacson, O. Relationships between stress protein induction and NMDA-mediated neuronal death in the entorhinal cortex. Exp Brain Res 1993;94:193-202.

51. Simpson JR, Isacson, O. Mitochondrial impairment reduces the threshold for in vivo NMDA-mediated neuronal death in the striatum. Exp Neurol 1993;121:57-64.

52. Frim DM, Simpson J, Uhler T, Short MP, Bossi SR, Breakefield XO, Isacson O. Striatal degeneration induced by mitochondrial blockade is prevented by biologically delivered NGF. J Neurosci Res 1993;35:452-8.

53. Bossi SR, Simpson JR, Isacson O. Age dependence of striatal neuronal death caused by mitochondrial dysfunction. NeuroReport 1993;4:73-6.

54. Frim DM, Short MP, Breakefield XO, Isacson O. Biological gene-product delivery to the brain: a protocol for retroviral gene transfer into cultured cells and intracerebral transplantation. NeuroProtocol 1993;3:63-8.

55. Frim DM, Uhler TA, Short MP, Ezzedine ZD, Klagsbrun M, Breakefield XO, Isacson O. Effects of biologically delivered NGF, BDNF, and bFGF on striatal excitotoxic lesions. NeuroReport 1993;4:367-70.

56. Frim DM, Yee WM, Isacson O. NGF reduces striatal excitotoxic neuronal loss without affecting concurrent neuronal stress. NeuroReport 1993;4:655-8.

57. Wullner U, Brouillet E, Isacson O, Young AB, Penney JB. Glutamate receptor binding sites change in MPTP-treated mice. Exp Neurol 1993;121:284-7.

58. Burns LH, Sato K, Wullner U, Isacson O. Intra-nigra infusion of AMPA attenuates dopamine-dependent rotation in the rat. NeuroReport 1993;4:1075-8.

59. Pakzaban P, Deacon T, Burns L, Isacson O. Increased proportion of AChE-rich zones and improved morphologic integration in host striatum of fetal grafts derived from the lateral but not the medial ganglionic eminence. Exp Brain Res 1993;97:13-22.

60. Brownell AL, Hantraye P, Wullner U, Hamberg L, Shoup T, Elmaleh DR, Madras B, Frim DM, Brownell GL, Rosen BR, Isacson O. PET- and MRI-based assessment of glucose utilization, dopamine receptor binding, and hemodynamic changes after lesions to the caudate-putamen in primates. Exp Neurol 1994;125:41-51.

61. Davar G, Kramer MF, Garber D, Roca AL, Andersen JK, Bebrin W, Coen DM, Kosz-Vnenchak M, Knipe DM, Breakefield XO, Isacson O. Comparative efficacy of gene delivery to mouse sensory neurons using herpes virus vectors. J Comp Neurol 1994;339:3-11.

62. Andersen JK, Frim DM, Isacson O, Breakefield XO. Herpes-virus mediated gene delivery into the rat brain: specificity and efficiency of the neuron-specific enolase promoter. Cell Mol Neurobiol 1994;13:503-15.

PART III: BIBLIOGRAPHY (172 Total: 104 Original, 66 Reviews/Chapters, 2 Books)

Original Reports (continued)

63. Uhler TA, Frim DM, Pakzaban P, Isacson O. The effects of mega-dose methylprednisolone and U-78517F on glutamate-receptor mediated toxicity in the rat neostriatum. Neurosurgery 1994;34:122-8.

64. Wullner U, Hantraye P, Brownell A-L, Pakzaban P, Burns L, Shoup T, Elmaleh D, Petto A, Spealman RD, Brownell GL, Isacson O. Dopamine terminal loss and onset of motor symptoms in MPTP-treated monkeys: a positron emission tomography study with 11C-CFT. Exp Neuro. 1994;126:305-9.

65. Frim DM, Uhler TA, Galpern W, Beal MF, Breakefield XO, Isacson O. Implanted fibroblasts genetically engineered to produce brain-derived neurotrophic factor prevent 1-methyl-4-phenylpyridinium toxicity to dopaminergic neurons in the rat. Proc Natl Acad Sci USA 1994;91:5104-8.

66. Andersen JK, Frim DM, Isacson O, Breakefield XO. Catecholaminergic cell atrophy in a transgenic mouse aberrantly overexpressing MAO-B in neurons. Neurodegeneration 1994;3:97-109.

67. Frim DM, Wullner U, Beal MF, Isacson O. Implanted NGF-producing fibroblasts induce catalase and modify ATP levels but do not affect glutamate receptor binding or NMDA receptor expression in the rat striatum. Exp Neurol 1994;128:172-80.

68. Pakzaban P, Geller A, Isacson O. Effect of exogenous nerve growth factor on neurotoxicity of and neuronal gene delivery by a herpes simplex amplicon in the rat brain. Human Gene Therapy 1994;5:987-95.

69. Andersen JK, Frim DM, Isacson O, Beal MF, Breakefield XO. Elevation of neuronal MAO-B activity in a transgenic mouse model does not increase sensitivity to the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Brain Res 1994;656:108-14.

70. Deacon TW, Pakzaban P, Burns LH, Dinsmore J, Isacson O. Cytoarchitectonic development, axon-glia relationships and long distance axon growth of porcine striatal xenografts in rats. Exp Neurol 1994;130:151-67.

71. Deacon TW, Pakzaban P, Isacson O. The lateral ganglionic eminence is the source of striatal phenotypes: neural transplantation and developmental evidence. Brain Res 1994;668:211-9.

72. Burns LH, Pakzaban P, Deacon TW, Brownell A-L, Tatter SB, Jenkins BG, Isacson O. Selective putaminal excitotoxic lesions in non-human primates model the movement disorder of Huntington disease. Neuroscience 1995;64:1007-17.

73. Pakzaban P, Deacon TW, Burns LH, Dinsmore J, Isacson O. A novel mode of immunoprotection of neural xenotransplants: masking of donor major histocompatibility complex class I enhances transplant survival in the CNS. Neuroscience 1995;64:983-96.

74. Tatter SB, Galpern WR, Hoogeveen AT, Isacson O. Effects of striatal excitotoxicity on Huntingtin-like immunoreactivity. NeuroReport 1995;6:1125-9.

75. Garcia AR, Deacon TW, Dinsmore J, Isacson O. Long-distance axonal growth from fetal porcine neural grafts in the excitotoxically lesioned adult rat cortex. Cell Transplant 1995;4:515-27.

76. Isacson O, Frim DM. Galpern, WR, Tatter SB, Breakefield XO, Schumacher JM. Cell-mediated delivery of neurotrophic factors and neuroprotection in the neostriatum and substantia nigra. Rest Neurol Neurosci 1995;8:59-61.

77. Isacson O, Deacon TW, Pakzaban P, Galpern WR, Dinsmore J, Burns LH. Transplanted xenogeneic neural cells in neurodegenerative disease models exhibit remarkable axonal target specificity and distinct growth patterns of glial and axonal fibres. Nature Med 1995;1:1189-94.

PART III: BIBLIOGRAPHY (172 Total: 104 Original, 66 Reviews/Chapters, 2 Books)

Original Reports (continued)

78. Dinsmore J, Deacon T, Ratliff J, Pakzaban, P, Jacoby, D. Isacson O. Embryonic stem cells differentiated in vitro as a novel source of cells for transplantation. Cell Transplant 1996;5:131-43.

79. Galpern WR, Frim DM, Tatter SB, Altar CA, Beal MF, Isacson O. Cell-mediated delivery of brain-derived neurotrophic factor enhances dopamine levels in a MPP+ rat model of substantia nigra degeneration. Cell Transplant 1996;5:225-32.

80. Galpern WR, Burns LH, Deacon TW, Dinsmore J, Isacson O. Xenotransplantation of porcine fetal ventral mesencephalon in a rat model of Parkinson's disease: functional recovery and graft morphology. Exp Neurol 1996;140:1-13.

81. Isacson O, Deacon TW. Specific axon guidance factors persist in the adult rat brain as demonstrated by pig neuroblasts transplanted to the rat. Neuroscience 1996;75:827-37.

82. Galpern WR, Matthews RT, Beal MF, Isacson O. NGF attenuates 3-nitrotyrosine formation in a 3-NP model of Huntington's disease. NeuroReport 1996;7:2639-42.

83. Deacon T, Schumacher J, Dinsmore J, Thomas C, Palmer P, Kott S, Edge A, Penney D, Kassissieh S, Dempsey P, Isacson O. Histological evidence of fetal pig neural cell survival after transplantation into a patient with Parkinson's disease. Nature Med 1997;3:350-3.

84. Haque N, Isacson O. Antisense gene therapy for neurodegenerative disease? Exp Neurol 1997;144:139-46.

85. Haque NSK, LeBlanc CJ, Isacson O. Differential dissection of the rat E16 ventral mesencephalon and survival and reinnervation of the 6-OHDA-lesioned striatum by a subset of aldehyde dehydrogenase-positive TH neurons. Cell Transplant 1997;6:239-48.

86. Costantini LC, Lin L, Isacson O. Medial fetal ventral mesencephalon: a preferred source for dopamine neuron grafts. NeuroReport 1997;8:2253-7.

87. Chen YCI, Brownell A-L, Galpern W, Keltner JR, Matthews RT, Isacson O, Rosen BR, Jenkins BG. Detection of dopaminergic neurotransmitter activity using pharmacologic MRI (phMRI): correlation with PET, microdialysis and behavioral data. Magn Res Med 1997;38:389-98.

88. Deacon T, Dinsmore J, Costantini LC, Ratliff J, Isacson O. Blastula-stage stem cells can differentiate into dopaminergic and serotonergic neurons after transplantation. Exp Neurol 1997;149:28-41.

89. Lin L, Leblanc C, Deacon T, Isacson O. Chronic cognitive deficits and amyloid precursor protein elevation after selective immunotoxic lesions of the basal forebrain cholinergic system. NeuroReport 1997;9:547-52.

90. Brownell A-L, Livni E, Galpern W, Isacson O. In vivo PET imaging in rat of dopamine terminals reveals functional neural transplants. Ann Neurol 1997;43:387-90.

91. Costantini LC, Chaturvedi P, Armistead DM, McCaffrey PG, Deacon TW, Isacson O. Distinct branching effects of a novel immunophilin ligand on dopaminergic neurons in culture, and neurotrophic actions after oral administration in an animal model of Parkinson's disease. Neurobiol Disease 1998;5:97-106.

92. Brownell A-L, Jenkins BG, Elmaleh DR, Deacon TW, Spealman, RD, Isacson O. Combined PET/MRS studies of the brain reveal dynamic and long-term physiological changes in a Parkinson's disease primate model. Nature Medicine 1998;4:1308-12.

93. LeBlanc CJ, Deacon TW, Whatley BR, Dinsmore J, Lin L, Isacson O. Morris water maze analysis of 192-IgG-saporin lesioned rats and porcine cholinergic transplants to the hippocampus. Cell Transplant 1999:8:131-42.

PART III: BIBLIOGRAPHY (172 Total: 104 Original, 66 Reviews/Chapters, 2 Books)

Original Reports (continued)

94. Deacon TW, Whatley B, LeBlanc C, Lin L, Isacson O. Pig fetal septal neurons implanted into the hippocampus of aged or cholinergic deafferented rats grow axons and form cross-species synapses in appropriate target regions. Cell Transplant 1999;8:111-49.

95. Brownell, A-L, Jenkins, B. and Isacson, O. Dopamine Imaging Markers and Predictive Mathematical Models for Progressive Degeneration in Parkinson's Disease. Biomed & Pharmacother 1999;53:131-40.

96. Kordower JH, Isacson O, Emerich, DF. Cellular delivery of trophic factors for the treatment of Huntingon's disease: is neuroprotection possible? Exp Neurol 1999;159:4-20.

97. Lin L, Georgievska B, Mattsson A, Isacson O. Cognitive changes and modified processing of amyloid precursor protein in the cortical and hippocampal system after cholinergic synapse loss and muscarinic receptor activation. Proc Natl Acad Sci USA 1999;96:12108-13.

98. Costantini LC, Jacoby DR, Wang S, Fraefel C, Breakefield XO Isacson O. Gene transfer to the nigrostriatal system by hybrid HSV/AAV amplicon vectors. Human Gene Therapy 1999;10:2481-94.

99. Chen YI, Brownell A-L, Galpern W, Isacson O, Bogdanov M, Beal MF, Livni E, Rosen BR, Jenkins BG. Detection of dopaminergic cell loss and neural transplantation using pharmacological MRI, PET and behavioral assessment. Neuroreport 1999;10:2881-6.

100. Haque NSK, Isacson O. Neurotrophic factors NGF and FGF-2 alter levels of huntingtin in striatal neuronal cell cultures. Cell Transplant 2000:in press.

101. Costantini LC, Isacson O. Immunophilin ligands and GDNF enhance neurite branching or elongation from developing dopamine neurons. Exp Neurol 2000:164;60-70.

102. Schumacher JM, Ellias SA, Palmer EP, Kott HS, Dinsmore J, Dempsey PK, Fischman AJ, Thomas C, Feldman RG, Kassissieh S, Raineri R, Manhart C, Fink JS, Isacson O. Transplantation of embryonic porcine mesencephalic tissue in patients with Parkinson's disease. Neurology 2000;54:1042-50.

103.Fink JS, Schumacher JM, Ellias SL, Palmer EP, Saint-Hilaire M, Shannon K, Penn R, Starr P, van Horne C, Kott HS, Dempsey PK, Fischman AJ, Raineri R, Manhart C, Dinsmore J, Isacson O. Porcine xenografts in Parkinson's disease and Huntington's disease patients: tentative outcomes. Cell Transplant 2000;9:273-8.

104. Freeman TB, Cicchetti F, Hauser RA, Deacon TW, Randall TS, Masiello I, Li X-J, Hersch SM, Nauert GM, Sanberg PR, Kordower JH, Saporta S, Isacson O. Survival of fetal striatal transplants in a patient with Huntington's disease, 2000:in press.

Reviews/Book Chapters

1. Isacson O, Brundin P, Dawbarn D, Kelly PAT, Gage FH, Emson PC, Björklund A. Striatal grafts in the ibotenic acid lesioned striatum. In: Bjorklund A, Stenevi U, eds. Neural grafting in the mammalian CNS. Amsterdam: Elsevier Science Publishers, B.V., 1985:539-49.

2. Brundin P, Isacson O, Gage FH, Stenevi U, Björklund A. Intracerebral grafts of neuronal cell suspensions. In: Bjorklund A, Stenevi U, eds. Neural grafting in the mammalian CNS. Amsterdam: Elsevier Science Publishers, B.V., 1985:51-9.

3. Emson PC, Dawbarn D, Rossor MN, Rehfeldt JF, Brundin P, Isacson O, Björklund A. Cholecystokinin content in the basal ganglia in Huntington's disease and expression of cholecystokinin-immunoreactivity in

PART III: BIBLIOGRAPHY (172 Total: 104 Original, 66 Reviews/Chapters, 2 Books)

Reviews/Book Chapters (continued)

striatal grafts to the ibotenic acid lesioned striatum of the rat. In: Vanderhaegen JJ, Crawley JN, eds. Ann NY Acad Sci USA 1985;448: 489-94.

4. Isacson O, Brundin P, Gage FH, Björklund A. Compensation for lesions induced changes in cerebral metabolism and behaviour by striatal neural implants in a rat model of Huntington's disease. In: Will, Schmitt, Dalrymple-Alford, eds. Brain plasticity, learning and memory, Plenum Press, 1985:519-35.

5. Gage FH, Björklund A, Isacson O, Brundin A. Uses of neuronal transplantation in models of neurodegenerative diseases. In: Das GP, Wallace RB, eds. Neural transplantation and regeneration. Springer-Verlag, 1986:103-24.

6. Isacson O, Björklund A, Dunnett SB. Conditions for neuronal survival and growth as assessed by the intracerebral transplantation technique in lesion models of the adult CNS. In: Althauser A, Seifert W, eds. Glial-neuronal communication in development and regeneration. Heidelberg: Springer Verlag, 1986:529-44.

7. Brundin P, Strecker RE, Lindvall O, Isacson O, et al. Intracerebral grafting of dopamine neurons: experimental basis for clinical trials in patients with Parkinson's disease. In: Cell and Tissue Transplantation into the Adult Brain. Ann NY Acad Sci USA 1987;495:473-97.

8. Isacson O, Pritzel M, Dawbarn D, Brundin P, et al. Striatal neural transplants in the ibotenic acid lesioned rat neostriatum: cellular and functional aspects. In: Cell and Tissue Transplantation into the Adult Brain. Ann NY Acad Sci USA, 198; 495:537-56.

9. Björklund A, Lindvall O, Isacson O, Brundin P, Wictorin K, Strecker RE, Clarke DJ, Dunnett SB. Mechanism of action of intracerebral neural implants: studies on nigral and striatal grafts to the lesioned striatum. Trends Neurosci 1987;10:509-16.

10. Björklund A, Brundin P, Isacson O. Neuronal replacement by intracerebral neural implants in animal models of neurodegenerative disease. In: Waxman SG, ed. Physiological basis for functional recovery in neurological disease. Advances in Neurology,NY: Raven Press,1988;47:455-92.

11. Gage FH, Brundin P, Strecker R, Dunnett SB, Isacson, O, Björklund A. Intracerebral neuronal grafting in experimental models of age-related motor dysfunction. In: Central Determinants of Age-Related Declines in Motor Function. Ann NY Acad Sci, 1988;575:383-93.

12. Dunnett SB, Isacson O, Sirinathsinghji DJS, Clarke DJ, Björklund A. Striatal grafts in the ibotenic acid lesioned neostriatum: functional studies. In: Gash DM, Sladek JR, eds. Neural Transplantation in the Mammalian CNS. Prog in Brain Res, Amsterdam:Elsevier 1988;78:39-47.

13. Wictorin K, Isacson O, Fischer W, Nothias F, Peschanski M, Björklund A Studies on host afferent inputs to fetal striatal transplants inthe excitotoxically lesioned striatum. In: Gash DM, Sladek JR, eds. Neural Transplantation in the Mammalian CNS, Prog in Brain Res, Amsterdam:Elsevier 1988;78:55-61.

14. Peschanski M, Nothias F, Dusart I, Isacson O, Roudier F. Reconstruction of the cytoarchitecture, synaptology and vascularization of the neuron-depleted thalamus using homotypic fetal neurons In: Bentivoglio M, Spreafico R, eds. Cellular Thalamic Mechanisms. Elsevier Science, 1988:543-54.

15. Clarke DJ, Dunnett SB, Isacson O, Bjorklund A. Striatal grafts in the ibotenic acid-lesioned neostriatum:ultrastructural and immunohistochemical studies. In: Gash DM, and Sladek JR, eds. Neural Transplantation in the Mammalian CNS Prog in Brain Res, Amsterdam:Elsevier, 1988;78:47-55.

16. Dunnett S, Isacson O. Trophic mechanisms are not enough. Trends Neurosci 1989;12:257.

PART III: BIBLIOGRAPHY (172 Total: 104 Original, 66 Reviews/Chapters, 2 Books)

Reviews/Book Chapters (continued)

17. Hantraye PH, Riche D, Maziere M, Maziere B, Loc'h C, Isacson O. Anatomical, behavioural and positron emission tomography studies of unilateral excitotoxic lesions of the baboon caudate-putamen as a primate model of Huntington's disease. In: Crossman AR, and Sambrook MA, eds. Neural mechanisms in disorders of movement. London: J Libbey & Co LTD, 1989:183-93.

18. Bjorklund A, Isacson O, Brundin P, Dunnett SB. Nigral and striatal grafts to the lesioned striatum:models of graft function. In: Gage FH, Privat A, Christen Y, eds. Intracerebral grafts and Alzheimer's disease. Springer-Verlag, 1989;4-20.

19. Peschanski M, Nothias F, Dusart I, Ontoniente B, Geffard M, Isacson O. Differential neural plasticity of diffuse monoaminergic and point-to-point systems as demonstrated by responses to target deprivation and fetal neural transplants.In:Gage FH, Privat A, Christen Y, eds. Intracerebral grafts and Alzheimer's disease. Springer-Verlag, 1989:177-88.

20. Dunnett SB, Björklund A, Brundin P, Isacson O, Gage FH. Transplantation of dopamine cell suspensions to the dopamine-depleted neostriatum. In: Stem G, ed. Parkinson's disease. London: Chapman Hall, 1990;239-65.

21. Sofroniew MV, Dunnett SB, Isacson O. Remodelling of intrinsic and afferent systems in neocortex with cortical transplants.In: Dunnett SB, Richards SJ, eds. Neural transplanation: from molecular bases to clinical application. Amsterdam: Elsevier 1990:313-320.

22. Isacson O, Arriagada PV, Hyman BT. Presence of morphologically distinct amyloid protein positive structures in non-demented elderly and Alzheimer's disease. Neurobiol Aging 1990;11:286.

23. Isacson O. Rules governing specificity and plasticity of neurons as demonstrated studies of neuronal transplants into the mature brain. Medecine/Sciences 1990;6:863-69.

24. Sofroniew MV, Svendsen CN, Isacson O. Changes in Basal Forebrain Cholinergic Systems Following Excitotoxic Cell Death In: Yoshida, ed. The Hippocampus and Cerebral Neocortex. 1991.

25. Isacson O, Hantraye P, Riche D, Schumacher JM, Maziere M. The Relationship Between Symptoms and Functional Anatomy in Chronic Neurodegenerative Diseases: From Pharmacological to Biological Replacement Therapy in Huntington's Disease. In: Intracerebral Transplantation in Movement Disorders. Restorative Neurology, Elsevier, 1991;4:245-58.

26. Riche D, Hantraye P, Isacson O, Maziere M. A Primate Model of Huntington's Disease: Unilateral Striatal Lesions and Neural Grafting in the Baboon. In: Bernardi O, et al., eds. Advances in Behavioral Biology. New York: Plenum Press, The Basal Ganglia III 1991;39:561-72.

27. Isacson O. Neurology and Neurobiology, Vol 60. Advances in Neural Regeneration Research, Seil F.J., ed. J. Neurosurg, 1991;75:491-92.

28. Frim DM, Andersen JK, Schumacher JM, Short MP, Isacson O, Breakefield X. Gene transfer into the central nervous system: neurotrophis factors.In: Moody TW, ed. Growth Factors, Peptides & Receptors. New York: Plenum Press, 1993;83-91.

29. Isacson O. On Neuronal Health. Trends in Neuroscience, 1993;16:306-308.

30. Isacson O. Clinical and Preclinical PET Correlates of Parkinsonism with 11C-WIN 35,428. Ann Neurol, 1994;35:377-378.

31. Isacson O. Fetal nerve cell transplantation: Advances in the treatment of Parkinson's disease. On the Brain, The Harvard Mahoney Neuroscience Institute Letter. 1994;3:4-5.

PART III: BIBLIOGRAPHY (172 Total: 104 Original, 66 Reviews/Chapters, 2 Books)

Reviews/Book Chapters (continued)

32. Pakzaban P, Isacson O. Neural xenotransplantation:reconstruction of neuronal circuitry across species barriers. Neuroscience, 1994;62:989-1001.

33. Tatter SB, Galpern WR, Isacson O. Mechanisms of neurotrophic factor protection against excitotoxic neuronal death. The Neuroscientist, 1995;1:286-97.

34. Breakefield XO, Sena-Esteves M, Pechan P, Kramm C, Yoshimoto Y, Lin Q, Davar G, Livermore J, Isacson O, Chiocca EA, Bohn M, Kaye E. Gene Therapy for the Nervous System-Status 1994, In: Genetic Therapy Fundacion BBV, 1995:503-25.

35. Isacson O. On behavioral effects and gene delivery in Parkinson's rat model. Science, 1995;269:856-57.

36. Isacson O. Review of "Neuroregeneration." J Chem Neuroanat 1995;9:149-52.

37. Freeman TB, Sanberg PR, Isacson O. Development of the human striatum: implications for fetal striatal transplantation. Cell Transplantation 1995;4:539-45.

38. Isacson O. Testimony for Hearing before the Special Committee on Aging, United States Senate: Breakthroughs in Brain Research: A National Strategy to Save Billions in Health Care Costs. June 27, 1995, Senate Hearing. 1995; Serial No. 104-230, 88-99.

39. Isacson O. Testimony for Hearing before the Subcommittee on Health and Environment of the Committee on Commerce US House of Representatives: Research Efforts with Respect to Combating Parkinson's Disease and other Neurological Disorders July 21, 1995. Serial No. 104-68, 1996;52-61

40. Isacson O, Kordower J. The neurobiological and clinical potential of neural cell transplantation: special issue based on the meeting of the American Society for Neural Transplantation, 1995 Cell Transplantation 1996;5:123-25.

41. Dinsmore JH, Pakzaban P, Deacon TW, Burns L, Isacson O. Survival of transplanted porcine neural cells treated with F(AB')2 antibody fragments directed against donor MHC class-I in a rodent model Transplant Proc 1996; 28:817-18.

42. Pakzaban P, Burns LH, Isacson O. Xenotransplantation, brain. In: Encyclopedia of Neuroscience. 1996;273:(CD Rom).

43. Isacson O. On the Causes and Treatments of Parkinson's Disease. In: Parkinson NPF Report, National Parkinson Foundation, Inc., 1996;Vol. XVI1, Issue 1: 8-11.

44. Galpern WR, Tatter S, Isacson O. Neurotrophic factor protection in models of neurodegeneration: implications for the treatment of neurodegenerative disorders In: Beal MF, Howell N, Bodis-Wollner I, eds., Mitochondria and Free Radicals in Neurodegenerative Diseases, New York: John Wiley & Sons Inc, 1997:557-83.

45. Isacson O, Pakzaban P, Galpern WR. Transplanting fetal neural xenogeneic cells in Parkinson's and Huntington's disease models. In: Freeman TB, Widner H, eds., Fetal Transplantation in Neurological Diseases. New Jersey:The Humana Press, 1997

46. Haque NSK, Borghesani P, Isacson O. Therapeutic strategies for Huntington's disease based on a molecular understanding of the disorder. Molec. Med Today. 1997;3:175-83.

47. Schumacher JM, Isacson O. Neuronal xenotransplantation in Parkinson's disease. Nature Med, 1997;3:474-75.

PART III: BIBLIOGRAPHY (172 Total: 104 Original, 66 Reviews/Chapters, 2 Books)

Reviews/Book Chapters (continued)

48 Isacson O, Haque N. Gene Therapy of Huntington's Disease. In: Chiocca AE, Breakefield X, eds., Gene Transfer and Therapy for Neurological Disorders. New Jersey:The Humana Press, 1997:423-40.

49. Isacson O, Breakefield XO. Benefits and risks of hosting animal cells in the human brain. Nature Med, 1997;3:964-69

50. Isacson O, Deacon T. Neural transplantation studies reveal the brain's capacity for continuous reconstruction. Trends Neurosci, 1997;20:477-82.

51. Sanberg P, Borlongan CV, Wictorin K, Isacson O. Fetal-tissue transplantation for Huntington's disease: Preclinical studies. In: Freeman TB, Widner H, eds., Cell Transplantation for Neurological Disorders. New Jersey:The Humana Press, 1998:77-93.

52. Isacson O, Pakzaban P, Galpern WR. Transplanting fetal neural xenogeneic cells in Parkinson's and Huntington's disease models. In: Freeman TB, Widner H, eds., Cell Transplantation for Neurological Disorders. New Jersey:The Humana Press, 1998:189-210.

53. Isacson O, Deacon T, Schumacher J. Immunobiology and neuroscience of xenotransplantation in neurological disease. In: Tuszynski M, Kordower JH, eds., CNS Regeneration, Basic Science and Clinical Applications, San Diego: Academic Press, 1998:365-87.

54. Emerich DF, Kordower JH, Isacson O. Cellular delivery of neurotrophic factors as a potential treatment for Huntington's disease. In: Tuszynski M, Kordower JH, eds., CNS Regeneration, Basic Science and Clinical Applications, San Diego: Academic Press, 1998:477-502.

55. Holm K, Isacson O. Factors intrinsic to the neuron can induce and maintain the ability for neurite outgrowth: a role for bcl-2? Trends Neurosci, 1999;22:269-73.

56. Boonman Z, Isacson O. Caspases in neuronal development and transplantation. Exp Neurol, 1999;156:1-15.

57. Costantini LC, Isacson O. Dopamine neuron grafts: development and molecular biology. In: di Porzio U, Pernas-Alonso R, Perone-Capano C, eds., Dopamine Neuron Development, Landes Company, Georgetown, 1999:123-37.

58. Isacson O, Costantini LC, Galpern WR. Molecules for neuroprotection and regeneration in animal models of Parkinson's disease. In: R Dean, D Emerich, eds., Innovative animal models of CNS diseases: From molecule to therapy, Humana Press, Totowa, NJ, 1999:187-207.

59. Isacson, O. The Neurobiology and Neurogenetics of Stem Cells. Brain Pathol. 1999;9:495-8.

60. Isacson, O. and Sladek, J. Cellular and Molecular Treatments of Neurological Diseases. Exp. Neurol. 1999;159:1-3.

61. Isacson, O. and Kang, U.J. The Potential of Gene Therapy for Treatment of Parkinson's Disease. In: Principles of Surgery for Parkinson's Disease and Movement Disorders, Krauss, K., Jankovic, J., Grossman, R. eds. Lippincott-Raven, 1999:in press.

62. Isacson O. (Discussant) Novartis Foundation Symposium, J. Goode, ed., 1999:in press.

63. Costantini LC, Bakowska JC, Breakfield XO, Isacson O. Gene Therapy in the CNS. Gene Therapy 2000;7:93-109.

PART III: BIBLIOGRAPHY (172 Total: 104 Original, 66 Reviews/Chapters, 2 Books)

Reviews/Book Chapters (continued)

64. Isacson O, Costantini L, Schumacher JM, Cicchetti F, Chung S, Kim K-S. Cell implantation therapies for Parkinson's disease using neural stem, transgenic or xenogeneic donor cells. In: Parkinson's Disease and Related Disorders, A Deutch ed., 2000:in press.

65. Isacson, O., van Horne, C., Schumacher, J.M., Brownell, A.-L. (2000) Improved surgical cell therapy in Parkinson's disease: physiological basis and new transplantation methodology. In: Parkinson's Disease, Advances in Neurology, D. Calne, ed. Lippincott Williams Wilkins, Philadelphia, PA, in press.

66. Emilien, G., Ponchon, M., Caldas, C., Isacson, O. and Maloteaux, M. (2000) Impact of genomics on drug discovery and clinical medicine. Quarterly Journal of Medicine (UK), 2000;93:391-423.

Books

1. Isacson O. Neural grafting in an animal model of Huntington's disease. Lund, Sweden: Lund University Library, 1987:1-188 (ISBN 91-7900-258-7).

2. Sanberg P, Wictorin K, Isacson O. Cell Transplantation for Huntington's Disease. Austin: R.G. Landes & Co, 1994 (ISBN 1-57059-079-6).